University of Alberta

Decapitation of trembling aspen (*Populus tremuloides*) to simulate aspen shoot blight [*Pollaccia americana*]

by

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Abstract

Integrated management of tree diseases requires an understanding of disease impact. For shoot blight [*Pollaccia americana* Ondrej] of trembling aspen (*Populus tremuloides* Michx.), this understanding will most likely come from studies which utilize a mechanical injury simulation technique to determine disease impact. Decapitation was tested to determine if it reproduced the effects of aspen shoot blight with fidelity. A field experiment compared aspen shoot blight to decapitation under plantation conditions, and a growth chamber experiment examined the relationship between disease and decapitation under different soil temperatures. Six populations of *P. tremuloides* and ten virulent isolates of *P. americana* were used to ensure that the results were not host genotype or pathogen isolate specific. In all cases the effects caused by shoot blight were reproduced by mechanical injury: decapitation caused a loss of current year's growth and alteration of form typical of aspen shoot blight infection. Some possible implications are discussed.

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List of Abbreviations and Symbols[†]

α – alpha, level of significance

ASB – aspen shoot blight

ASC – axillary shoot counts

C – control

CMI – Commonwealth Mycological Institute

CPA – chick pea agar

D – decapitation

F – field experiment

GC – growth chamber experiment

I – injury treatment

IPM – integrated pest management

MEA – malt extract agar

n/a – [data] not available

OSB – oriented strand board

p – significance probability; $p^{\sim}$ – Holm-adjusted significance probability

PAR – photosynthetically active radiation

SL – seedlot

SLM – shoot length measurement

spp. – species, plural

TNC – total non-structural carbohydrates

UAMH – University of Alberta Microfungus Collection and Herbarium

vs. – versus

WA – water agar

[†] Note: metric units are given in SI (Système international d unités) convention, and journal abbreviations follow BIOSIS® format. Neither type of abbreviation/symbol is included in this list.

Chapter 1: General Introduction

1.1 Trends in Aspen Resource Management

Species of the genus *Populus* are dominant on 9.2% (20 million hectares) of the productive forest in Canada (Peterson and Peterson 1992) and represent over 3 billion m^3 of volume (Canadian Council of Forest Ministers 2003). Of the poplar species native to Canada, trembling aspen (*Populus tremuloides* Michx.) is the most widespread (Zasada et al. 2001) and has the most resource potential (Peterson and Peterson 1992). In the prairie provinces (Alberta, Saskatchewan, and Manitoba), trembling aspen accounts for over 80% of the poplar volume (Peterson and Peterson 1992) and 37% of the total gross merchantable volume (Brandt et al. 2003). This resource is invaluable: aspen provide erosion control, habitat, carbon sinks, viewsheds, and a host of other non-timber benefits in addition to major timber uses such as oriented strand board (OSB), pulp and paper, lumber, and specialty products (Peterson and Peterson 1992; Zasada et al. 2001). Recent trends in Alberta have indicated that utilization of aspen by the forest product industry is substantial and increasing; in 1997 10 million m^3 of aspen were harvested (43% of the total harvest), an increase of 800% over the previous 15 years (Zasada et al. 2001).

In the past, aspen harvests have been almost exclusively from extensively managed stands. However, most managers of the aspen resource now expect that intensively managed stands will become necessary in the near future to increase the supply of aspen fibre and/or lumber (Navratil et al. 1990; Zasada et al. 2001). This move towards more intensive management will likely involve practices such as density management, stand rejuvenation, and genetic improvement (Navratil et al. 1990), all of which are designed to reduce the rotation age at which aspen is most economically harvested. Aspen has historically been harvested at around 65 to 85 years (Kabzems et al. 1986), although the culmination of mean annual increment (MAI) can occur at rotation ages of around 30 years in extensively managed stands (Bella and De Franceschi 1980). Intensive forestry offers the opportunity to produce sawlogs within 20 to 30 years, and to harvest aspen for fibre in less than 20 years (Stanturf et al. 2001). Such attempts at

short rotation forestry will undoubtedly increase the financial investment of the forestry sector, and thus make periodic losses in growth potential due to diseases such as aspen shoot blight far more important than earlier considered (Newcombe et al. 2001).

1.2 Aspen Shoot Blight and Other Shoot Blights of Poplar

Poplar shoot blights are diseases of poplars found in North America (e.g. Dance 1961a), Europe (e.g. Weisgerber 1968), Asia (e.g. Wu and Sutton 1995), and the Indian sub-continent (e.g. Kahn and Misra 1989). In North America, shoot blights affect the aspen poplars (section *Populus*, formerly *Leuce* Duby) as well as the cottonwoods (section *Aigeiros* Duby), balsam poplars (section *Tacamahaca* Spach) and their intra- and inter-sectional hybrids (Newcombe 1996). The name of the pathogen causing aspen shoot blight on *Populus tremuloides* Michx. is thought to be *Venturia tremulae* Aderhold var. *grandidentatae* Morelet (anamorph *Pollaccia americana* Ondrej), although there is considerable debate over the proper taxonomy.

1.2.1 Taxonomy

The specific identity of the agent causing shoot blight of the aspen poplars (section *Populus*) is uncertain. Early work was conducted in Europe; in 1937 Baldacci and Cifferri described the pathogenic conidiogenous fungi on the genus *Populus* as *Pollaccia radiosa* (Lib.) Bald. et Cif. (Dance 1959). Shortly thereafter, Servazzi erected another species, *Pollaccia elegans* Serv. (Dance 1961a). Unfortunately, the distinction between these species was confused through mutual synonymy via *Napicladium tremulae* (Frank) Sacc. and *Fusicladium radiosum* (Lib.) Lind. (Dance 1961a). Dance, working in Ontario, helped to clarify the situation two decades later by connecting *P. radiosa* to *Venturia tremulae* Aderhold (1959) and *P. elegans* to *Venturia populina* (Vuill.) Fabric. (1961a). Barr's (1967) review of the *Venturiaceae* collected in North America listed *Venturia tremulae* as a synonym for *Venturia macularis* (Fr.) E. Müller et von Arx, and maintained the anamorph/teliomorph connection between *V. macularis* and *P. radiosa* as well as

between *V. populina* and *P. elegans*. This taxonomy was generally well accepted, with most studies of shoot blights on aspen poplars using the names *V. tremulae* (or *V. macularis*) and/or *P. radiosa* to describe the pathogen (Ginzburg 1961; Hanso and Tamm 1973; Kechel 1983; Marinkovic et al. 1982; Sivanesan 1974; Siwecki 1968; Sutton 1973; Weisgerber 1968,1969).

In 1972, Ondrej reviewed the taxonomy of *Pollaccia* species on *Populus*. Ondrej (1972) separated the *Pollaccia* on the European aspens (*P. tremula* L., *P alba* L.) from that on the North American aspens (*P. tremuloides* Michx., *P. grandidentata* Michx.). In doing so he erected a new species, *Pollaccia americana* Ondrej, to describe the North American fungus and renamed the shoot blight pathogen on *P. alba* (*Cladosporium ramulosum* Desm.) as *Pollaccia ramulosum* (Desm.) Ondrej. The binomial *P. radiosa* was thus reserved for the shoot blight pathogen on *P. tremula* (Ondrej 1972).

Morelet, having knowledge of Barr's contention that European and North American specimens of *V. macularis* were "identical" (Barr 1967, p. 810), helped to clarify the apparent contradiction in taxonomy introduced by Ondrej's (1972) study which separated the anamorphs by continent. Morelet reclassified the *Venturia* on section *Populus* by removing the synonymy between *V. tremulae* and *V. macularis*, and by introducing a new species with two varieties, *Venturia viennotii* Morelet var *viennotii* and *V. viennotii* Morelet var *levispora* (Morelet 1985). In addition, he separated *V. tremulae* into three varieties: *V. tremulae* Aderhold var. *tremulae* Morelet, *V. tremulae* Aderhold var. *populi-albae* Morelet, and *V. tremulae* Aderhold var. *grandidentatae* Morelet (Morelet 1985). Concomitant cultural biology studies connected *V. tremulae* var. *tremulae* to *Pollaccia radiosa* (Lib.) Bald. et Cif., *V. tremulae* var. *populi-albae* to *P. radiosa* (Lib.) Bald. et Cif. var. *populi-albae* Morelet, and *V. tremulae* var. *grandidentatae* to *P. radiosa* (Lib.) Bald. et Cif. var. *lethifera* (Peck in Sacc.) Morelet (Morelet 1985, 1987). Morelet considered *Pollaccia americana* Ondrej to be synonymous with the latter variety of *P. radiosa* (Morelet 1985).

Ascospore inoculation studies led Morelet to suggest that *V. macularis* and *V. viennotii* were incapable of causing infections on aspen (Newcombe 1996). Later work

suggested an inability of these pathogens to produce conidia under conditions suitable for conidiogenesis in other *Venturia* species (Morelet 1987). Morelet interpreted these findings as further evidence for the removal of synonymy between *V. macularis* and *V. tremulae*. If he was correct, his findings explain the inconsistency between Barr's and Ondrej's findings: *V. macularis* is non-pathogenic and does not produce conidia, therefore Ondrej's by-continent division of the pathogenic anamorphs does not necessarily apply. Ondrej did not make teliomorph connections, which is unfortunate, as this would help to sort out the true relationship between the sexual and asexual fungi.

In 1989, Funk described *Pollaccia borealis* Funk on *Populus tremuloides* in British Columbia and the Yukon Territory (Funk 1989). This pathogen causes a disease (purple-brown leaf spot) that can easily be differentiated from aspen shoot blight on the basis of symptom expression. More recently, Kasanen, Hantula, and Kurkela reported a species of *Venturia* on blighted shoots of *Populus tremula* in Finland that was morphologically and genetically distinct from *V. macularis* (Kasanen et al. 2001). Additional genetic profiling led Kasanen et al. to conclude that these isolates were not a new species, but rather *Venturia tremulae* Aderhold var *tremulae* Morelet (Kasanen et al. 2004). This finding provides additional evidence for Morelet's (1985) argument against the synonymy of *V. macularis* and *V. tremulae* as reported by Barr (1968) and Sivanesen (1974).

Not all pathologists and mycologists, however, agree with Morelet's taxonomy. Recent North American field guides (e.g. Hiratsuka et al. 1995), and surveys of forest health (e.g. Brandt et al. 2003) list *Venturia macularis* as the agent causing shoot blight on trembling aspen. As such, the taxonomy of aspen-infecting *Venturia* in North America can be considered still in flux.

1.2.1.1 Agent Identity

Given the taxonomic uncertainty surrounding the *Venturia/Pollaccia* on *Populus*, the true identity of the fungal agent used in this study is not certain. Blenis and Chow (2001) report the name of the aspen shoot blight pathogen collected in Alberta and British

Columbia as *Pollaccia americana* Ondrej. My decision to use the same name for these specimens is based on the following points of logic: 1) while Morelet's *Venturia tremulae* Aderhold var *grandidentatae* is the only known North American *Venturia* causing shoot blight on *Populus tremuloides*, the description and identification of the North American *Venturiaceae* may be incomplete; 2) all work was conducted with the conidiogenous state, and no ascospores have been observed in culture; 3) Ondrej's anamorph classification included collections from Western Canada and Morelet's did not; and 4) the description of conidia given by Ondrej is a good fit to those used in this study.

Given the uncertainty in shoot blight taxonomy, and to aid future understanding, most of isolates used in the following experiments have been preserved at the University of Alberta Microfungus Collection and Herbarium (UAMH). Accession numbers are listed in a table in Chapter 2.

1.2.2 Lifecycle

The biology of aspen shoot blight (ASB) is reasonably well known. The pathogenic conidial stage is produced in the spring from fungal mycelia which has over-wintered in diseased tissue or has developed from the germination of ascospores (Weisgerber 1969). Rain-splash disseminated conidia (Dance 1961b) infect the tips of succulent shoots (Blenis and Chow 2001) causing leaf spotting and curling and necrosis of the shoot tip (the "shepherd's crook" symptom). Olivaceous and necrotic lesions appear predominantly on young leaves. These lesions often expand throughout the leaf lamina and into the petiole and ultimately onto the shoot which, eventually, dies. Secondary infections may be caused by subsequent generations of the anamorph produced in infected tissue during the spring and early summer (Dance 1961b; Ginzburg 1961). Ascospores, produced in the spring from over-wintered pseudothecia, are thought to function primarily as wind-disseminated dispersal propagules (Weisgerber 1969). However, recent evidence from a study of shoot blight of *Populus tremula* in Finland (Kasanen et al. 2004) suggests that ascospores may be produced further into the summer

than suggested by Weisgerber (1969) and could thus be a more important source of primary infections that previously thought.

1.2.3 Host Specificity

Dance suggested that the pathogenic *Venturia* species were separated by host preference: *V. populina* infecting the poplars of sections *Tacamahaca* and perhaps *Aigeiros*, and *V. tremulae* limited to the *Populus* poplars (Dance 1961a). This specificity is reflected in a taxonomic review of poplar-infecting *Venturia* (Newcombe 1996) and other recent literature (e.g. Kasanen et al. 2001). However, evidence exists that section specificity in *Venturia/Pollaccia* is not absolute. Barr (1967) reported *V. macularis* on poplars of the section *Aigeiros* and Ondrej (1972) found *P. elegans* on *P. grandidentata*. The cross-inoculations required to determine whether *Venturia/Pollaccia* spp. are indeed section specific have not been performed.

In addition to Funk's (1989) discovery of a new *Pollaccia* species on section *Populus*, new *Venturia* species have recently been described by Morelet (1993; *Venturia mandshurica* Morelet) on poplars of sections *Aigeiros* and *Tacamahaca* (Wu and Sutton 1995), and by Newcombe (2003; *Venturia inopina* G. Newc.) on *Tacamahaca* poplars and *Tacamahaca* x *Aigeiros* hybrids. The discovery of additional poplar-infecting *Venturia* species is likely, and it is probable that our understanding of the poplar-infecting members of this genus will evolve from that of wide-ranging section-specific pathogens to pathogens with a more local distribution and limited host range (Newcombe 2003).

1.2.4 Disease Impact

The current paradigm in disease management, known as integrated pest management (IPM), is a decision support system based on applications of ecological knowledge about the identity, life history, population dynamics, and potential impact of a causal agent (Norris et al. 2003). In addition to knowledge of the biology and potential impact of a pest, IPM requires an understanding of potential control strategies and the economic, social, legal, and environmental considerations attached to their use (Norris et al. 2003).

This information is used to delimit thresholds beyond which damage is sufficient to justify control measures such as host manipulation or pest reduction (Norris et al. 2003). For aspen shoot blight, improving host genetic resistance is often suggested as the best approach for reducing disease-induced losses (Dance 1961b; Kasanen et al. 2004; Kechel 1983; Marinkovic et al. 1982; Siwecki 1968; Weisgerber 1968). Before such programs are deployed, however, impact studies are required to determine whether the impact of ASB is sufficient to justify the associated economic, social, and environmental costs.

To date, few studies have examined the impact of aspen shoot blight. Early on, Dance described the pathogen causing ASB as "ubiquitous and destructive" (Dance 1961a, p. 883). Field observations led him to suggest that the disease had severe effects on seedlings and suckers (Dance 1961b). Dance suggested that shoot blight infections in young plantations would result in reduced regeneration success, sapling deformation, and a year or so delay in stand establishment (Dance 1961b). These suggestions were later evaluated by Anderson and Anderson (1980) who studied a local epidemic of aspen shoot blight in a two-year old burned-over aspen stand in Minnesota. Anderson and Anderson (1980) found that 96% of regenerating aspen were infected in the first year of study and that most (80%) of the spring growth and some (36%) of a second flush were blighted. Infections in the following two years were slightly less destructive, with around 80% of aspen infected and an average tissue loss of 44 to 49%. At this level of infection, secondary growth and secondary infections were rare. In the second year of infection, Anderson and Anderson (1980) protected some trees with the fungicide benomyl (methyl 1-[butyl-carbamoyl]-2-benzimidazole carbamate) to determine the growth loss due to disease. They reported an average annual leader loss of 7.6 cm for that year and the next, and 10.2 cm in the fourth and fifth years. In addition to leader loss, they reported a degradation of form in diseased trees due to a competition between multiple leaders (Anderson and Anderson 1980).

Gross and Basham (1981) reported the results of disease surveys across 45 regenerating aspen stands in Ontario in 1977. They found ASB in 96% (43/45) of stands sampled, with light infections (6-25% of trees affected) in four stands and trace infections

(<6% of trees affected) in 39 stands. These investigators suggested that the most important effect of ASB on the host was a loss of leader length of about one-third and a resultant loss of form; they speculated that stand stagnation and butt log distortion was possible in stands with repeated severe infections. Similar results were reported by Perala (1984) who monitored biotic injuries to aspen (insect and disease) in two stands in Minnesota over seven years: loss of leader length in ASB-infected trees was common, and in some cases was an impediment to tree class differentiation.

A recent study of aspen health in the Canadian prairie provinces suggested that most aspen in this region are healthy. Across 85 permanent sample plots examined, less than 10% of trees had been affected by aspen shoot blight (Brandt et al. 2003). Despite this overall bill of health, a dieback was evident in the transitional forest which separates the grassland from the boreal (Brandt et al. 2003). ASB may have played a role in this mortality, likely in combination with other biotic and abiotic agents (Brandt et al. 2003). Further research is required to evaluate this contention.

The few studies that have been conducted on the impact of ASB on trembling aspen have shown that the immediate effects are a loss of succulent tissue including the leader, and a subsequent change in crown form. The long term impacts of ASB, however, remain unknown. While some have speculated, none of the studies reported above followed infected trees from the time of infection to a time of stand maturation. In order to understand the effect of ASB on mature stands and on aspen resource use, longer-term experimental approaches are required. Until such long and medium-term studies have been conducted, the impact of ASB on *P. tremuloides* will remain unknown.

1.3 Study Rationale and Research Objectives

Long-term experimental studies of shoot blight impact have not been successful, as investigators have been unable to reliably produce infection in plantations, and reference trees in infected natural stands often become blighted. Although reference trees may be

protected by fungicides, this approach requires that a suitable product is found[1] and that questions of its efficacy, phytotoxicity, and safety are addressed. Research in controlled environments is possible. However, a true understanding of disease impact requires research under field conditions. As such, the development of a disease simulation technique is a priority.

Two experiments were designed to evaluate the degree of similarity in the response of *P. tremuloides* to decapitation and ASB. The first tested the simulation technique against aspen shoot blight on seedlings from four seedlots under field conditions. The second tested the simulation technique on two clones grown at three soil temperatures under controlled-environment conditions. My working hypothesis was that aspen would respond to decapitation in a similar manner as it would respond to ASB. Furthermore, I expected that injury treatments would induce a change in morphology that would not be seen in control trees. Secondary objectives were to provide some indication of the effect of host genotype (seedlot or clone) and soil temperature on disease development.

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[1] Benomyl, used to protect trees by Anderson and Anderson (1980), is no longer registered for use in Canada (Pest Management Regulatory Agency 2004).

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Chapter 2: Decapitation of Trembling Aspen (*Populus tremuloides*) to Simulate Aspen Shoot Blight [*Pollaccia americana*]

2.1 Introduction

Pollaccia americana Ondrej, the anamorph of *Venturia tremulae* var. *grandidentatae* Aderhold., is the causal agent of aspen shoot blight ("shepherd's crook") on aspen poplars of the section *Populus* in North America (Morelet 1985; Ondrej 1972). In *Populus tremuloides* Michx. (trembling aspen) this disease is associated with the formation of a necrotic crook on the terminal shoot. Although the impact of shoot blight on *P. tremuloides* has been speculated about by a number of authors, a coherent understanding of the effects of aspen shoot blight (ASB) on *P. tremuloides* is still beyond reach. Suggested impacts vary from a loss of current growth on young trees (Anderson and Anderson 1980) to reduced regeneration success (Dance 1961), stand stagnation (Gross and Basham 1981), and inhibited tree differentiation (Perala 1984). These predictions remain speculative, however, as no long term impact studies have been carried out.

In the context of integrated pest management (IPM), an understanding of disease impact is necessary for development of prudent control strategies. This knowledge is required, first and foremost, when attempting to determine the threshold level of damage beyond which control strategies are economically sound. Without such knowledge, resources spent on the development of appropriate control measures may be misallocated if potential impacts are determined to be less than the cost of control.

Studies on the impact of ASB have proven difficult. Long-term *in-situ* studies have been limited by an inability to select appropriate uninfected control trees and maintain them in an uninfected state, and controlled experiments have been hampered by difficulties in successfully inoculating trees in the field (Blenis and Greidanus, unpublished data). These problems have led to the idea that it may be possible to

simulate disease with wounding, thus allowing long-term field impact studies to be conducted.

Simulating herbivory and/or disease by mechanical damage has several advantages. Simulations provide the advantages of requiring less time for treatment application and allowing the investigator greater control over (and quantification of) the magnitude of damage (Baldwin 1990). Simulations also uncouple herbivore choice/host resistance from the effect of injury by allowing investigators to randomly assign damage to experimental units rather than trying to separate the effects of observed damage from those of predisposing factors (Baldwin 1990). These theoretical advantages have led many entomologists, mammalogists, and plant physiologists to use simulation techniques to study the effect of biotic defoliation in herbaceous plants (e.g. Cranshaw and Radcliffe 1980), shrubs (e.g. Raworth and Clements 1996), and trees (e.g. Dolch and Tscharntke 2000; Edenius 1993; Hall and Ferree 1976; Hjältén et al. 1993). A number of these artificial defoliation studies have been conducted on *Populus* species (e.g. Bassman et al. 1982; Reichenbacker et al. 1996) including *Populus tremuloides* (Hart et al. 1999; Oiser and Lindroth 2001).

In addition to those who have used artificial defoliation as a simulation technique, a number of researchers have directly compared wounding to the biotic defoliator it aims to simulate. Ostile and Pedigo (1984) were able to build on previous work by Poston et al. (1976) to successfully develop a simulation technique for mimicking defoliation of soybean (*Glycine max* (L.) Merr.) by lepidopteran insects. Recent research by Srinivas et al. (2001), however, shows that mechanical injury cannot serve as a surrogate for feeding damage caused by a coleopteran pest of soybean. Attempts at developing insect damage simulations in other systems have also been a mixture of success and failure. Insect defoliation has been successfully simulated in lodgepole pine (*Pinus contorta* Doug. ex Loud.) (Britton 1988), ponderosa pine (*Pinus ponderosa* Doug. ex P. et C. Lawson) (Sanchez-Martinez and Wagner 1994), balsam fir (*Abies balsamea* (L.) Mill.) (Piene and Little 1990), and Douglas-fir (*Psuedotsuga menziesii* (Mirb.) Franco) (Chen et al. 2002). Simulations have not been successful for insect defoliation of wheat (*Triticum aestivum*

L.) (Capinera and Roltsch 1980), alfalfa (*Medico sativa* L.) (Havličková 1982), birch (*Betula pendula* Roth) (Hartley 1988; Hartley and Lawton 1987), and Scots pine (*Pinus sylvestris* L.) (Lyytikainen-Saarenmaa 1999). Comparisons between mammalian grazing and artificial defoliation (clipping) of grama (*Bouteloua curtipendula* (Michx.) Torr.) (Reardon et al. 1974), rye-grass (*Lolium perenne* L.) (Howe et al. 1982), and sweet thorn (*Acacia karroo* Hayne) (Teague 1988) have not been successful.

The ability of mechanical injury to simulate biotic defoliation depends on the fidelity of the simulation technique to the pattern, severity, and rate of damage caused by the biotic defoliator (Ostile and Pedigo 1984; Poston et al. 1976). Though by no means a universal postulate, theory suggests that successful simulation techniques are most likely to be developed where the goals of the research are to understand the effect of a biotic defoliator on the growth and morphology of a plant (Baldwin 1990). Investigators examining chemical responses to feeding (e.g. Hartley 1999; Hartley and Lawton 1988) or other aspects of trophic ecology (e.g. induced resistance; Srinivas et al. 2001) are unlikely to find a suitable simulation technique (Baldwin 1990). Some other considerations include the magnitude of the simulated agent's role in nutrient turnover via saliva (Reardon et al. 1972) or feces (Lerdau 1996), as well as the ability of the agent to stimulate plant responses via damage cues, such as cell wall fragments (Ryan et al. 1986), or endogenous growth regulators.

In contrast to the frequent use of artificial defoliation by researchers in other fields, this type of simulation has not been well utilized by plant pathologists. Pioneering work was conducted by Hendrix et al. (1965), who compared mechanical defoliation to stripe rust [*Puccinia striiformis* West.] on wheat, and by Piening and Kaufmann (1969), who compared manual leaf removal to net blotch [*Dreschlera teres* (Shoemaker) Sacc.] on barley (*Hordeum vulgare* L.). Lockwood et al. (1977) then adapted the mechanical injury techniques used by others (agrologists and entomologists) studying soybean defoliation to determine the impact of brown spot [*Septoria glycines* Hemmi] and bacterial blight [*Pseudomonas gycinea* Coerper] of soy, while Zilberstein et al. (1985) introduced the use of chemical simulation techniques by applying magnesium chlorate to

wheat as a simulator of post-anthesis leaf blotch [*Septoria tritici* Rob. ex Desm.]. Later, Bahl and Kahl (1995) used both mechanical injury and salicylic acid to simulate the compounding effects of unspecified pathogens on tobacco plants (*Nicotiana tabacum* L.) subjected to air pollution treatments. Most recently, Lusso and Pascholati (1999) applied either silicon carbide, an abrasive, or *Bipolaris maydis* (Nisik.) Shoemaker to maize (*Zea mays* L.) to quantify peroxidase activity after injury or infection.

Of these six studies, only three actually compared the simulation technique to the effect of the pathogen in an effort to develop a reliable proxy. Hendrix et al. (1965) found that mechanical defoliation was unable to account for the effects of *P. striiformis* on wheat. Piening and Kaufmann (1969) found that the fidelity of defoliation to disease impact was dependent on the plant tissue removed/infected, as well as the fertilizer regime. The most recent test, that by Zilberstein et al. (1985), found that wounding slightly overestimated impact but was still useful in screening cultivars for tolerance to speckled leaf blotch.

Initial studies of wounding as a disease simulation technique in our laboratory focused on determining the type of wound which most appropriately simulated the pattern and severity of damage evident in trees afflicted by aspen shoot blight. Although a number of simulation techniques caused damage similar to that caused by ASB (Blenis and Greidanus, unpublished data), decapitation with a razor blade was found to be the most reproducible.

During initial investigations, *P. tremuloides* responded differently to wounding depending on whether trees were grown in the greenhouse or in the field (Blenis, unpublished data). This observation suggests that environmental conditions such as soil temperature may be important when studying the response of aspen to leader damage such as that caused by aspen shoot blight. Cool soils reduce the leaf area and shoot height of aspen (Landhäusser and Lieffers 1998, Landhäusser et al. 2001, Wan et al. 1999), and may affect the synthesis and transport of cytokinins (Skogerbø and Måge 1992; Tachibana 1988; Tromp and Ovaa 1994). These growth regulators have been

implicated in regulating correlative inhibition via apical dominance (Cline 1994; Cline and Dong-Il 2002; Tamas 1995).

With the foregoing considerations in mind, the overall objective of this study was to assess the utility of decapitation as a research technique for studying the impact of aspen shoot blight. Two experiments were designed to evaluate the degree of similarity in the response of *P. tremuloides* to decapitation and ASB. Specifically, it was expected that aspen would respond to decapitation in a similar manner as it would to ASB. Furthermore, it was expected that injury treatments would induce a change in morphology that would not be seen in control trees. Secondary objectives were to provide some indication of the effect of host genotype (seedlot or clone) and soil temperature on disease development.

2.2 Materials and Methods

2.2.1 Experiment One: An assessment of decapitation as an ASB simulation technique under field conditions

The first experiment tested decapitation against aspen shoot blight on seedlings from four seedlots under field conditions at Edmonton, Alberta (approximate location 54°33'N-113°28'W). Treatment structure was a 4 x 3 factorial with four seedlots and three injury treatments: inoculation with a *P. americana* conidial suspension, decapitation with a razor blade, and an uninjured control. Design structure was a randomized complete block with four blocks. One quarter of the seedlings within each seedlot were randomly assigned to each of the blocks, and within each block-seedlot combination, groups of three trees (henceforth referred to as triplets) were selected based on similar appearance. Control, ASB, and decapitation treatments were then randomly assigned to trees in each triplet.

2.2.1.1 Host Culture

Dormant 1+0 over-wintered seedlings from four seedlots were obtained from nurseries in western Canada (Table 2.1). Seedlings from each seedlot were immediately assigned to

blocks, with blocks differentiated by planting date. They were then placed in cold storage (-3 ˚C +/- 3 ˚C) upon receipt and remained in cold storage until 2 hours before they were planted.

Differences in planting date combined with differences in seedlot arrival date resulted in differences in cold storage duration, though all seedlings were planted within 66 days (minimum 27 days) of arrival. On each of the planting days, seedlings were planted in 15 cm fibre pots (Kord standard; Kord Products Inc., Brampton, ON) containing Metromix® 290 growing media (Terra-Lite 2000 series; WR Grace and Company, Ajax, ON), amended with 2 g L^{-1} Nutricote® 60 day slow release 14-14-14 fertilizer (7% NO_3-N, 7% NH_4-N, 14% P_2O_5, 14% K_2O; Plant Products Company Ltd., Brampton, ON) and 0.03 g L^{-1} Chelated Micronutrient Mix (2.1 ppm Fe, 0.6 ppm Mn, 0.12 ppm Zn, 0.03 ppm Cu, 0.39 ppm B, 0.018 ppm Mb; Plant Products Company Ltd., Brampton, ON) (Thomas 1996). After planting, seedlings were fertilized on day 1 and day 15 with 300 mL 0.4% 10-52-10 fertilizer (5% NO_3-N, 5% NH_4-N, 52% P_2O_5, 10% K_2O plus micronutrients; Plant Products Company Ltd., Brampton, ON), with no further fertilization. Planted seedlings were placed in cold frames on the roof of the Agriculture-Forestry Building at the University of Alberta campus (separated by block), and allowed to break dormancy and grow for 28 days.

2.2.1.2 Pathogen Production

Ten isolates of *P. americana* originating in Alberta and British Columbia were selected (Table 2.2), based on consistent sporulation and pathogenicity in preliminary trials. In each pathogen production cycle (once per block), single-spore cultures of these 10 isolates growing on malt-extract agar (MEA; Tuite 1969, Medium No. 128) were removed from cold storage. Three potato dextrose agar (PDA; Difco™ potato dextrose agar) plates were produced for each isolate by transferring plugs of mycelium from the MEA plates to PDA using a multi-point subculture technique (Blenis and Chow 2001). PDA plates were incubated in the dark for 3 days at room temperature, and then placed on a light bench under Gro-Lux© wide spectrum fluorescent bulbs (Sylvania; Osram

GmbH, Munich, Germany) for 7 days. Fungal colonies from all three PDA plates were then used to make initial conidial suspensions by shaking the colonies from each plate in 50 mL centrifuge tubes containing 15 mL sterile distilled water. Suspensions were checked for conidia under a light microscope and 1 mL of the most concentrated solution was then spread onto each of four chick pea agar (CPA; Tuite 1969, Medium No. 49) plates. CPA plates were incubated under light for 7 to 14 days, depending on the time required for sufficient levels of sporulation.

The number of conidia growing on one of four CPA plates per isolate was periodically assessed in the second week of incubation. Assessments were originally conducted microscopically. Five 5 mm diameter mycelial plugs were transferred to a 50 mL centrifuge tube containing 2 mL distilled water. This tube was shaken and the concentration of the resulting conidial suspension was determined using a Neubauer-ruled haemocytometer (Tuite 1969). This process was later replaced by macroscopic inspection of plates once the approximate relationship between colony colour/density and conidia production were understood. When sporulation was greater than approximately 10^5 conidia mL^{-1}, conidial suspensions (inoculum) for each isolate were produced from the remaining three CPA plates. Agar from each plate was cut into 9 cm^2 pieces, placed into a 250 mL screw-capped Erlenmeyer flask containing 60 mL sterile distilled water, and shaken for 60 seconds to produce a conidial suspension (Blenis and Chow 2001). Conidial suspensions were strained through polyester wool to remove agar and were then bulked by isolate. Approximately 150 mL of inoculum was produced per isolate.

For each isolate, inoculum concentrations were determined using a haemocytometer. The four isolates with the least populous conidial suspensions were discarded. Inoculum produced from the six remaining isolates were mixed together to yield 900 mL. The conidial concentration of the mixed isolate solution was determined using a haemocytometer.

No attempt was made to equalize conidial concentration among blocks or between isolates. Doing so would 1) require a reduction in the overall concentration to that of the least populous, or 2) require a reduction in volume of inoculum via centrifugation, and 3)

increase the likelihood of conidia germinating in solution due to the increased handling time. Blenis and Chow (2001) showed that concentrations of 10 x 10^4 *P. americana* conidia mL^{-1} were sufficient to cause severe infection on aspen seedlings. At over twice this rate (Table 2.3), inoculum concentrations used in all four blocks were assumed to be non-limiting.

2.2.1.3 Inoculation

On the 28th day of growth, all seedlings within a block were brought into the greenhouse at the Agriculture-Forestry Building. The greenhouse was maintained at 21/16 °C (day/night) with a 17 hour photoperiod and approximately 60% relative humidity. Irradiance varied with cloud cover, but averaged about 400 µmol photons m^{-2} s^{-1} PAR (photosynthetically active radiation) at the tree canopy level.

Selected seedlings were placed into a spray chamber and a hand-pump sprayer was used to spray the top 10 cm of foliage with inoculum suspensions until run-off (approximately 20 mL) (Blenis and Chow 2001). An acetate shield was used to protect all but the top 10 cm of foliage from inoculum. Blocks 1 through 3 were inoculated with conidial suspensions of six isolates, but Block 4 received a conidial suspension of only five isolates due to plate contamination and/or poor growth of the other five isolates (Table 2.3). Eight water agar plates (WA; Tuite 1969, Medium No. 258) were sprayed at the same time as seedlings to check germination of conidia, and one seedling per seedlot was sprayed with the water used to make spore suspensions to provide an uninfected-yet-sprayed comparison. These seedlings and water agar plates were not subject to analysis; germination was high for all blocks (Table 2.3) and no symptoms developed on any of the seedlings sprayed only with water.

Immediately after inoculation, inoculated seedlings were randomly assigned to one of three wood and clear polyethylene boxes which were placed in the greenhouse to increase relative humidity. Shade cloth was draped over top of the boxes, reducing incident light to about 300 µmol photons m^{-2} s^{-1} PAR. Boxes were vented and remisted with distilled water daily to reduce heat and gas build-ups. Trees were incubated in the

boxes for 3 days. During this period, the temperature within the boxes varied from 18 °C to 32 °C. The other two-thirds of the seedlings (those assigned to decapitation and control) remained in the greenhouse during the three day incubation period; these seedlings were not placed in boxes.

2.2.1.4 Plantation Construction and Transplantation

A 0.33 hectare area of agricultural land at the Edmonton Research Centre was selected on the basis of appropriate drainage, previous use (fallow), and sufficient distance from other *P. tremuloides* stands. The area was fenced to exclude wildlife. Four blocks were created, each containing 12 plots (one for each seedlot-injury combination). Within each plot, the position of 12 sample trees was marked with pin flags. Plots were spaced 2 m apart to allow for mechanical weed control, and blocks were aligned upon a suspected soil moisture gradient and spaced by 4 m to allow for equipment passage.

On the 31st day of growth, seedlings from all three injury treatments were transported to the field site and transplanted into 12 previously flagged plots. Holes of approximately 30 cm diameter and 30 cm depth were dug at the location of each flag in each of the plots. Holes were filled with water. Fibre pots containing seedlings were cut in four locations, and the portion of the pot above the soil line was removed. The modified pot was then placed in the hole, and the field soil-water slurry was used to fill in the gaps between the pot and the side of the hole. All transplanted seedlings were watered immediately after transplantation in order to reduce transplant stress and to initiate the process of pot decomposition. Further watering occurred as necessary.

2.2.1.5 Decapitation

Decapitated seedlings were wounded on the 42nd day of growth. This day, 11 days following transplanting, was chosen to correspond with the approximately 14 day time period required for inoculated trees to form necrotic crooks (Gelhorn, unpublished data). The leader of each seedling was cut half way along the internode between the fifth and sixth partially expanded (leaf midrib and veins visible) leaf below the apex by severing the shoot with a single horizontal cut (Figure 2.1). Razor blades were surface sterilized

in a solution of 70% ethanol (v/v) and allowed to air dry between seedlings; they were replaced after 12 seedlings.

2.2.1.6 Measurements

Inoculation success was quantified by recording disease incidence (the number of trees in a group with ASB infection severe enough to kill the infected leader and produce a characteristic "shepherd's crook") at 14, 21, and 28 days following inoculation (Figure 2.2). In order to quantify the effect of injury treatments on trees, measurements of shoot morphology were scheduled to occur at the end of the growing season in 2002 and again in 2003, and leaf area and above-ground biomass were intended to be measured after the 2003 growing season. Unfortunately, changing land use plans required the dismantling of field plots in the spring of 2003, thus precluding a second season of measurement. As such, only the details pertaining to morphological measurements are presented here.

The lengths of all terminal shoots (leaders) and first order branches were measured to the nearest mm, and the number of first order branches per tree was determined. First order branches are those that originate on the terminal and are synonymous with second order shoots. Branches were categorized based on ontogeny, with those branches arising from the main stem below the terminal bud scar classified as laterals (Figure 2.3). In contrast to lateral branches, those branches arising from the axils of leaves on the current year's growth were classified as axillaries. Axillary branches arose without a season of dormancy between bud formation and shoot expansion; they are sylleptic (Wu and Hinckley 2001). In contrast, lateral branches were a mixture of sylleptic and proleptic (arising after a latent season of dormancy) shoots (Wu and Hinckley 2001). Use of the terms "lateral" and "axillary" was chosen in order to prevent confusion between current year and previous year sylleptics.

Not all trees in each plot were measured, as some trees became unsuitable for shoot length analysis because either 1) the tree was dead; 2) the leader had been killed unintentionally, usually by insects or mechanical damage; 3) for inoculated trees, infection was not sufficient to kill the leader; 4) ASB infection occurred on trees not

treated with *P. americana*; 5) additional infections on ASB trees occurred on branches; 6) a failure to correctly determine which shoot would be the leader caused the decapitation treatment to be placed (inappropriately) on a lateral branch; or 7) the tree was alive but did not show any first order branch development. Axillary branches were counted (but not measured) on trees culled only for criterion 5. As such, sample sizes for each block-seedlot-injury combination varied and were dependent on the type of measurement: shoot length measurement (SLM) or axillary shoot count (ASC) (Table 2.4).

2.2.1.7 Analysis

2.2.1.7.1 Disease Incidence

Trees were considered successfully infected if the shoot tip was killed. Data were analysed by Friedman's non-parametric test for related samples (Conover 1980) due to heterogeneity of variance among seedlots. α was set at 0.05.

2.2.1.7.2 Leader Length

Trees within each block-seedlot-injury combination were regarded as samples and averaged prior to statistical analysis. Data were analyzed using SAS MIXED procedure (SAS version 8.0, SAS Institute, Cary, N.C.), as a randomized block with the model $Y_{ijk} = B_i + I_j + L_k + IL_{jk} + \varepsilon_{ijk}$ where:

Y_{ijk} = the response of seedlings within the i^{th} block and k^{th} seedlot to the j^{th} injury treatment;

B_i = the random effect of the i^{th} block, i = 1 to 4;

I_j = the fixed effect of the j^{th} injury treatment (ASB, decapitation, or control), j = 1 to 3;

L_k = the random effect of the k^{th} seedlot, k = 1 to 4;

IL_{jk} = the random effect of the interaction between the j^{th} injury and the k^{th} seedlot; and

ε_{ijk} = the residual.

Injury effects were partitioned into two orthogonal contrasts: ASB and decapitation vs. control, and ASB vs. decapitation. α was set at 0.05.

2.2.1.7.3 Branch Length

Measurements of branch length for each tree were unit scaled by dividing the length of each branch by the sum of the lengths of all first order branches. This was done to provide information on the pattern of growth exhibited by each tree, and to limit the morphological variation inherent in the seedlings. Leader length was excluded from the sum of the branch lengths, as its inclusion would introduce an artifact of treatment into the measurement of response: the leader length was manipulated in two of three injury treatments. After length for each branch was determined as a proportion of total branch length, branches of each type (lateral or axillary) were ranked by their magnitude, and values for trees within each block-seedlot-injury combination were averaged. The proportional length of the largest two branches of each type (lateral and axillary) were then analysed irrespective of position (Figure 2.3).

Statistical analysis of proportional branch length was undertaken using the same model as was used for the analysis of leader length. Heteroscedascity among residuals was modeled using proc MIXED, and the same orthogonal *a-priori* contrasts were performed as for the leader length data. These analyses were conducted separately for each of the largest two lateral and axillary shoots. Attained levels of significance for each response variable were adjusted using the Bonferroni-Holm method (Holm 1979; Westfall et al. 1999) to prevent inflation of the family-wise error rate beyond $\alpha = 0.05$ across multiple tests; these values are denoted $p^{\sim}$. Analyses of the random factor seedlot and seedlot-by-injury interaction effects were undertaken using the GLM procedure (SAS version 8.0, SAS Institute, Cary, N.C.). While both the GLM and MIXED procedures allow for the incorporation of random effects in the analysis, the former provides a better test for random effects if there are few levels of the random factor (Littell et al. 1996).

2.2.1.7.4 Axillary Shoot Development

Axillary shoot counts for each block-seedlot-injury combination were analysed in two ways. The average number of axillary shoots per tree was computed, and the data were analysed using the model and procedures discussed above for leader length data. In

addition, the number of trees which developed axillary shoots were determined for each block-seedlot-injury combination, and then analysed by using the CATMOD procedure (SAS version 8.0, SAS Institute, Cary, N.C.) to perform an asymmetrical log-linear analysis (Kennedy 1992) on the presence or absence of axillary shoots. Data were first analysed for block-by-injury interaction. After finding no significant interaction ($p = 0.4920$), data were combined across blocks. Component likelihood ratio chi-squares (L^2) were calculated to show the effects of seedlot, injury, and their interaction for orthogonal contrasts among injury treatments (ASB and decapitation vs. control and ASB vs. decapitation). α was set at 0.05.

2.2.2 Experiment Two: Controlled environment assessment of decapitation as an ASB simulation technique

The second experiment provided a repeat of the field study under controlled-environment conditions and allowed the effect of soil temperatures to be incorporated. Hypothesis testing was accomplished by growing two clones of *P. tremuloides* at three soil temperatures which would be commonly encountered in the boreal forest (Hogg and Lieffers 1991), and subjecting these trees to the three injury treatments (ASB, decapitation, and control). Three water baths (blocks) were assigned to each temperature, and within each bath, two stecklings were planted for each combination of clone and injury; hence, a total of 108 trees were used.

2.2.2.1 Host Culture and Temperature Treatments

Dormant 1+0 stecklings from two clones (Table 2.1) were placed in a cold storage facility (-2 °C) in Drayton Valley, Alberta. On the planting day, stecklings were removed from cold storage, transported to Edmonton and, within 2 hours, placed in a cold room (4 °C) to thaw for 2 hours before planting. At the time of arrival in Edmonton, the lateral buds on both clones were swollen but the scales had not yet separated.

Stecklings were planted into modified 20 cm diameter Eezy-Gro™ self-watering pots (Apollo Plastics Ltd., Mississauga, ON). Modification included removing a self-watering wick and sealing a side drain hole. Pots were then filled with a 5 cm layer of

granite grit (Number 4 Masco Granite Grit; International Marble and Stone Company Ltd., Creston, BC) which was overlaid by a false bottom and vented by a 15 cm vinyl hose (Johnson Industrial Plastics, Edmonton, AB). Trees were planted into a 1:1 (v/v) mixture of Metromix® 290 growing media (Terra-Lite 2000 series; WR Grace and Company, Ajax, ON) and sifted sand which had been placed above the false bottom in each pot. The soil surface was then covered by a layer of the light coloured granite grit to increase albedo and pot weight.

Stecklings were grown in a growth chamber with an 18 hour photoperiod (350 - 400 μmol m^{-2} s^{-1} PAR immediately above the soil surface), 60% relative humidity, and a 22/16 °C day/night air temperature. This chamber was equipped with nine water baths designed to maintain soil temperatures at 6 °C, 14 °C, and 20 °C. These insulated plastic boxes (90 x 90 x 20 cm) were connected to a closed water circulation system (Landhäusser et al. 2001). Water was cooled in a common circulating cooler, and then pumped through insulated hoses to insulated storage reservoirs (one per temperature) which were each connected to three water baths. A steady flow of cold water was maintained at approximately 12 L min^{-1} from storage reservoirs to water baths. Water was pumped into the water bath through a perforated hose attached to the bottom of the bath and warmer surface water was collected via an overflow pipe which returned water to the reservoir. The temperature of water entering each storage reservoir was controlled by adjusting the flow rate of water from the common cooling unit. Water flow was completely shut off to the 20 °C reservoir; the associated water baths remained dry throughout the experiment to allow air temperatures to maintain the soil temperature at 20 °C. Once the optimal flow rate for each temperature was determined, no further adjustments to flow were made.

Twelve potted stecklings were placed in each water bath, and water level was adjusted to ensure that all the pot area below the soil line was below water but that the water level was not so high as to flood the trees. Water bath, soil, and air temperatures were checked four times weekly with handheld thermometers. Stecklings were watered as needed to maintain field capacity, and fertilized once a week with 100 mL of 15-30-15

fertilizer (7% NO_3-N, 7% NH_4-N, 30% P_2O5, 15% K_2O plus micronutrients; Plant Products Company Ltd., Brampton, ON) at the following concentrations: weeks 0 to 2 – 0.1%, weeks 3 to 7 – 0.2%, weeks 8 to 12 – 0.4%. Drainage water which accumulated in the false bottom was removed by syringe suction via the installed vinyl hose 4 hours after each watering and 24 hours after each fertilization.

2.2.2.2 Pathogen Production and Inoculation

Five isolates of *P. americana* originating in Alberta were selected (Table 2.2) and cultured to produce inoculum as described above. Approximately 170 mL of inoculum from each isolate was combined to yield about 850 mL of a mixed-isolate inoculum with a concentration of 18×10^4 conidia mL^{-1} (Table 2.3).

Stecklings were inoculated on the 22nd day of growth. Two randomly-selected stecklings from each clone (four trees in total) were inoculated in each bath. The inoculation order (clone and water bath) was random, and the inoculation procedure was exactly as described above. Conidia germination was above 90% on all eight WA plates (Table 2.3).

Following inoculations, all stecklings (inoculated or not) were covered with clear polyethylene bags (Scienceware Polyethylene Utility Bags; Fisher Scientific, Nepean, ON) which contained a water-saturated paper towel. Bags were loosely tied to allow air exchange while maintaining high humidity, and were removed and replaced daily to dissipate any gas build-ups. Bags were removed 3 days following inoculation.

2.2.2.3 Decapitation

Stecklings were decapitated on the 36th day of growth. Two stecklings per clone were randomly selected in each water bath; the decapitation order (clone and water bath) was random. The decapitation procedure was exactly as described above.

2.2.2.4 Measurements

Disease incidence was assessed 14 and 21 days following inoculation. Inoculated trees without crooks after 21 days were reinoculated. Trees with crooks on any shoot other than the leader were identified as having lateral infections.

In order to understand the response of trees to treatments, measurements of morphology similar to those conducted in the field experiment were planned. These measurements would have assessed the length of the leader and the number and length of lateral and axillary branches. In the weeks following inoculation 36% (13/36) of inoculated trees unexpectedly developed lateral infections. These infections precluded meaningful branch length measurement, and the loss of experimental units prevented analysis of branching patterns. Hence, only leader length and the presence or absence of axillary shoots on the leader were analysed.

2.2.2.5 Analysis

2.2.2.5.1 Disease Incidence

Inoculated trees within each temperature-clone combination were coded as infected (1) or not (0) and averaged over blocks prior to analysis of the proportion of seedlings infected for each combination of temperature and clone. Preliminary analysis indicated that the temperature effect was consistent across clones (i.e. there was no clone-by-temperature interaction). The GLM procedure was then used to perform an analysis of covariance with the following model: $Y_{ij} = TX_i + C_j + \varepsilon_{ij}$ where:

Y_{ij} = the proportion of infected trees from the j^{th} clone grown at the i^{th} soil temperature;

TX_i = the fixed covariate effect of the i^{th} temperature (6 °C, 14 °C, or 20 °C), i = 1-3;

C_j = the random effect of the j^{th} clone, j = 1-2; and

ε_{ij} = the residual and error term.

α was set at 0.05.

2.2.2.5.2 Leader Length

Leader lengths were analysed as a split plot with temperature as the whole-plot factor and injury and the injury-by-temperature interaction as the subplot factors. Orthogonal polynomial contrasts (Steel et al. 1997) were used to determine if there was a linear or quadratic relationship between temperature and leader length. As before, the effects of injury were partitioned into orthogonal contrasts: ASB and decapitation vs. control, and ASB vs. decapitation. α was set at 0.05.

2.2.2.5.3 Axillary Shoot Development

As in the field study, the CATMOD procedure was used to perform an asymmetrical log-linear analysis (Kennedy 1992) on the presence or absence of axillary shoots. Temperature and injury were included as factors in the analysis. Clone and block were not included due to insufficient degrees of freedom, so data were averaged over clone and block prior to analysis. α was set at 0.05.

2.3 Results

2.3.1 Disease Incidence

2.3.1.1 Field Experiment

Disease incidence in the field differed significantly ($p < 0.001$) among seedlots (Figure 2.4), with across block averages of 100% (48/48), 77% (37/48), 60% (28/47), and 56% (27/48) for seedlots 1 to 4 respectively.

2.3.1.2 Growth Chamber Experiment

Disease incidence also differed significantly ($p = 0.0192$) between the two clones tested in the growth chamber, being 83% (15/18) for clone 1 and 44% (8/18) for clone 2. These values include one tree from clone 1 which was successfully reinoculated; reinoculations

were unsuccessful for thirteen other trees (clone 1 and 2). The percent of trees with ASB-killed leaders increased significantly ($p = 0.0156$) with soil temperature (Figure 2.5, $R^2 = 0.9389$). The interaction between temperature and clone was non-significant ($p = 0.9558$).

2.3.2 Effect of Injury Treatments

2.3.2.1 Leader Lengths

2.3.2.1.1 Field Experiment

Average leader lengths (and 95% confidence intervals) of field grown trees were 14.7 cm (+/- 3.4), 12.9 cm (+/- 3.4), and 26.5 cm (+/- 3.4) for diseased, decapitated, and control trees respectively (Figure 2.6). Leader lengths were similar between diseased and decapitated trees, with a mean difference of 1.8 +/- 6.9 cm ($p = 0.3942$, Figure 2.6). When compared to the controls, injuries (ASB and decapitation) reduced leader lengths by 12.6 +/- 4.2 cm ($p = 0.0003$; Figure 2.6).

2.3.2.1.2 Growth Chamber Experiment

Diseased, decapitated, and control trees in the growth chamber had average leader lengths of 11.0 cm (+/- 3.1), 8.6 cm (+/- 2.7), and 28.3 cm (+/- 2.7), respectively (Figure 2.7). Leader lengths were similar between diseased and decapitated trees, with a mean difference of 2.4 +/- 3.8 cm ($p = 0.2153$; Figure 2.7). On average, ASB and decapitation reduced leader length by 18.5 +/- 3.1 cm ($p < 0.0001$; Figure 2.7). There was a significant overall effect of temperature on leader length ($p = 0.0364$; Figure 2.8), and a linear trend was detected ($p = 0.0213$). The quadratic temperature effect and the temperature-by-injury interaction were not significant ($p = 0.1061$ and 0.1293, respectively).

2.3.2.2 Branch Growth Pattern

The branch growth pattern was only examined in the field experiment. This parameter refers to the percentage of 2002 length apportioned to each of the first order branches with the exception of the terminal (leader). Although the majority of branch growth for all trees was in the lateral branches (Figure 2.9), axillary branch growth was the most sensitive indicator of injury to the leader (Figure 2.10). Axillary branches accounted for a significantly larger portion of the total branch length in trees that were decapitated or diseased than in uninjured controls (Figure 2.10). Whereas the longest axillary shoot accounted for 12.5% of the total branch length on injured trees, it accounted for only 2% of the branch length on control trees. Similarly, the second longest axillary branch accounted for 5.7% and 1.1% of the total branch length for injured and uninjured trees, respectively. In contrast, laterals accounted for more branch growth on control than on injured trees; the longest two lateral branches accounted for 45.0% and 24.2% of the branch growth on control trees and 35.8% and 20.0% of the branch growth on injured trees. All differences between injured and control trees were significant (Figure 2.10). Only small and statistically insignificant differences in growth pattern existed between diseased trees and decapitated trees (Figure 2.11).

Differences in growth pattern existed among seedlots. Among the four seedlots, there were differences in the length of the longest lateral branch and the two longest axillary branches (Table 2.5). For each of the four response variables analysed, the seedlot-by-injury interaction was non significant (Table 2.5), indicating that the effect of injury treatments was consistent across seedlots.

2.3.2.3 Axillary Shoot Development

2.3.2.3.1 Field Experiment

Between 34% and 94% of ASB-infected trees produced axillary branches (Figure 2.12a), with an average of 1.8 (+/- 0.3) branches produced per tree (Figure 2.13). Similarly, 32% to 92% of decapitated trees developed axillary shoots (Figure 2.12a), with an average of

1.6 (+/- 0.3) axillary shoots per tree (Figure 2.13). Injury differences between diseased and decapitated trees were statistically insignificant for both the percent of trees with axillary shoots (Table 2.6a) and the number of axillary shoots produced per tree ($p = 0.2219$). Significantly fewer control trees developed axillary shoots than did injured trees (Figure 2.12b; Table 2.6b), and the average number of axillary shoots per tree was lower in control trees (0.6 +/- 0.3) than in injured trees (Figure 2.13; $p < 0.0001$). There were significant differences among seedlots in the number of axillary shoots per tree ($p < 0.0001$) and the presence/absence of axillary shoots (Table 2.6a,b). In neither case was there a significant interaction between seedlot and injury ($p = 0.2428$ for axillary shoots per tree; see Table 2.6a,b for presence/absence of axillary shoots).

2.3.2.3.2 Growth Chamber Experiment

In the growth chamber, 67% to 100% of ASB-infected trees and 45% to 50% of decapitated trees developed axillary shoots (Figure 2.14a). Differences in shoot development between ASB and decapitation were non-significant (Table 2.7a). None of the control trees developed axillary shoots, and as such, injury treatments were significantly different from controls (Figure 2.14b; Table 2.7b). Temperature did not have a significant effect on axillary shoot development (Table 2.7a,b; Figure 2.14a,b), and there was no significant interaction between temperature and injury (Table 2.7a,b).

2.4 Discussion

Inoculations were successful in causing disease on two-year old trembling aspen. ASB caused a mean 11.7 cm and 17.3 cm loss of leader length in the field and growth chamber, respectively. These values are similar to those reported by Anderson and Anderson (1980). In addition to this loss of leader length, ASB affected the form of trees by causing a loss of correlative inhibition of axillary branches. A loss of correlative inhibition (apical dominance) results in a proliferation of branches near the apex of the tree, often leading to multiple leaders and a loss of optimal form (Anderson and Anderson 1980).

The percentage of trees infected varied among the four seedlots (Figure 2.4) and two clones (Figure 2.5) studied. The most likely explanation is that this variation among clones/seedlots represents differences in genetic resistance to ASB. However, since the consistency of cultural factors during the first year of growth (at different nurseries) is unknown, it may be possible that differences in container type, fertilization, greenhouse environments, and lifting dates may explain some of this variability in disease incidence. As such, further testing with stock grown in an entirely consistent manner would be required to rule out differences in nursery culture.

Differences in resistance to *Venturia/Pollaccia* infections have been shown among 9 to 24 clones of *P. tremula* (Kechel 1983; Weisgerber 1968), and among open pollinated *P. tremula* from five provenances (Kechel 1983). Resistance has also been shown in hybrids within the section *Populus* (Kechel 1983; Siwecki 1968; Weisgerber 1968), including *P. tremula* x *P. tremuloides* (Kechel 1983). The only study that examined the resistance of native *P. tremuloides* to ASB (Blenis and Chow 2001) found no significant differences in resistance among the five clones tested, despite finding a significant clone-by-isolate interaction.

Should differences in disease incidence among trembling aspen be shown to result from genetic factors and not cultural ones, it would make possible suggestions by some (e.g. Kasanen et al. 2004) that breeding for resistance to ASB will become an important aspect of aspen culture if disease impact justifies control measures. However, further research is required to confirm the existence, and determine the extent and heritability, of ASB resistance.

Soil temperatures had an effect on disease incidence. For both clones, the frequency of stem necrosis increased with increasing temperature (Figure 2.5). This suggests that low soil temperatures decreased tree susceptibility.

Although the effects of soil temperature on the development of foliar diseases have not been well studied, the effect of cold air temperatures on host resistance has been investigated in several studies of psychrophillic fungi of cereal crops. These studies suggest that the process of cold hardening at temperatures between 2 °C and 6 °C may

confer resistance to infection (Gaudet and Chen 1987; Nakajima and Abe 1996). This resistance may be a function of an increased production of pathogenesis-related (PR) proteins (Gaudet et al. 2000), lipid transfer proteins (Gaudet et al. 2003), or plant defensins (Gaudet et al. 2003), all of which are thought to be part of plant defense barriers (García-Olmedo et al. 1998). Activation of the genes coding for these protein transcripts is thought to be triggered by an increase in the cytoplasmic concentration of carbohydrates during the process of hardening (see Gaudet et al. 1999 for a model of hexose-sensing).

Like plants which are hardened in cool air temperatures, aspen grown in cool soils also accumulate carbohydrates in the cytoplasm. Landhäusser et al. (2001) found that a reduction in the growth rate of aspen grown in cool soils was associated with an increased concentration of total non-structural carbohydrates (TNC) in the leaves and for the whole plant. As growth reductions at cool soil temperatures were evident in this study (Figure 2.8), and because carbohydrate accumulation has been linked to the production of plant defense proteins (Gaudet et al. 1999), a possible increase in TNC may explain the decreased disease incidence observed in trees grown at 6 ˚C over those grown at 14 ˚C or 20 ˚C. An alternative explanation would be that trees grown at 6 ˚C had less succulent tissue available at the time of inoculation, and thus difficulties in obtaining sufficient levels of infection were a consequence of a reduced target size.

Regardless of the mechanism involved, this difficulty in obtaining infection on trees grown in cool soils limited the sample size available to test the effect that the 6 ˚C soil temperature had on axillary shoot development in diseased and decapitated trees. Though this reduction in sample size is regrettable, there is no evidence to suggest that soil temperature will affect the consistency of the relationship between ASB and decapitation.

This study may have underestimated the growth loss caused by cool soils, as shown by Landhäusser et al. (2001), Landhäusser and Lieffers (1998), and Wan et al. (1999), because soil temperatures in the cool soil treatments were not absolutely consistent. Three days were required to establish the desired temperatures once trees

were placed in the baths; this resulted in trees spending the first 72 hours after planting in warmer-than-specified soils. Furthermore, equipment failure caused the loss of cooling water from all three 14 °C water baths on two separate occasions. These equipment failures lasted for a period of up to 56 and 25 hours, respectively. A further disruption of 4.5 hours was required for preventative maintenance in both the 6 °C and 14 °C water baths. The maximum temperature reached was 20 °C for the 14 °C water baths in all three interruptions, and 15 °C for the 6 °C water bath during the preventative maintenance. The time-temperature relationship during each episode was unknown. Wan et al. (2001) found significant increases in root and shoot water potentials and net carbon assimilation in as little as 30 minutes following the transfer of aspen grown in 5 °C soils to 20 °C soils. Temperature increases from 10 °C to 20 °C caused shoot water potential to become less negative, but did not significantly alter root water potential or carbon assimilation (Wan et al. 2001). Because the temperature fluctuations due to equipment failure were not well quantified in my study, the magnitude and persistence of the effects they had on tree growth is not known.

Irrespective of the environmental conditions tested, comparisons of diseased trees with decapitated trees showed that this mechanical injury technique has the potential to be an effective tool for simulating the losses due to aspen shoot blight. In both experiments, decapitation caused a slightly larger but statistically insignificant loss of current year's length growth than did ASB (Figure 2.6; Figure 2.7). This difference in severity is likely to be acceptable for most applications of the simulation technique. The shoot development patterns for diseased and decapitated trees were similar (Figure 2.11; Figure 2.12a; Figure 2.13; Figure 2.14a), as both injuries caused a loss of apical dominance resulting in a proliferation of axillary branches which was not seen in control trees (Figure 2.10; Figure 2.12b; Figure 2.13; Figure 2.14b). This relationship was consistent across the four seedlots tested; there was no seedlot-by-injury interaction despite significant differences in morphology among trees from the various seedlots (Table 2.5; Table 2.6a,b). Thus, the relationship between ASB and decapitation appears to be robust with respect to genotypic and environmental variability.

That the loss of the terminal leader resulted in the production of axillary branches is hardly unexpected. Physiologists have long known that removal of the shoot apex of many plant species causes a loss of correlative inhibition (Cline 2000). The most common explanation for this phenomenon is one of interplay between repressive apically-derived auxin and promotive root-derived cytokinin (Cline and Dong-Il 2002), though some debate still exists among hormone physiologists (see Davis 1995 and Trewavas 1981). As both decapitation and ASB kill the shoot apex, a loss of apical dominance is not surprising.

The more interesting question is whether decapitation fully accounts for the changes to the host tree induced by ASB. In theory, answering this question would require an understanding of the infection biology of *P. americana*. Literature in this area is sparse, although some lessons may be gleaned from research into the infection biology of two closely related species, *Venturia nashicola* Tanaka et Yamamoto (pear scab) and *Venturia inaequalis* (Cooke) G. Wint. (apple scab). In both species a germ tube from a conidium contacts the cuticle and produces an appressorium coated in a polysaccharide slime thought to aid in adhering to the cuticle (Park et al. 2000). Infection pegs develop from the appressorium and eventually penetrate the cuticle, giving rise to subcuticular hyphae which develop in the pectin layers and congregate at the junction between epidermal cells (Park et al. 2000). Neither these hyphae nor any other fungal body enters the epidermal cytoplasm (Park et al. 2000). As such, pectinases and other fungal enzymes are thought to be important in infection (Park et al. 2000).

If the infection biology of *P. americana* is similar to that of its teliomorphic congeners, it is unlikely that any simulation technique could accurately mimic all aspects of the infection and disease development process. Instead, only the most salient features should be considered: 1) shoot blight produces a necrotic crook usually two weeks following inoculation (Gelhorn, personal observation), and 2) only the succulent apex of any shoot will become infected (Blenis and Chow 2001) so growth losses are restricted to those internodes represented by the youngest five or so leaves (Gelhorn, unpublished data). These features are amenable to simulation by decapitation, thus decapitation meets

the pattern and severity requirements stipulated by Poston et al. (1976), Ostile and Pedigo (1984), and Baldwin (1990) for developing an effective simulation technique.

Decapitation cannot account for the gradual rate of shoot death observed in inoculated trees, nor for any endogenous growth substances (damage cues or "hormones") or elicitor/receptors produced by the fungus or the plant in response to infection. Because of these limitations, it is unlikely to be useful in simulating ASB for the purposes of understanding resistance. Likewise, it will not be a useful tool for determining the epidemiology of disease.

In summary, a mechanical injury technique was tested to determine if it adequately simulated the effect of aspen shoot blight [*Pollaccia americana*] on trembling aspen (*Populus tremuloides*). This study was of a short term nature (two experiments, each over one growing season), but compared ASB to decapitation at different soil temperatures and over a range of host populations amongst which there was significant morphological variation. Furthermore, this study involved the use of ten virulent isolates of *P. americana* to ensure that the results were not isolate-specific. In all cases, the effect caused by ASB was reproduced with fidelity: decapitation caused a loss of current year's growth and alteration of form typical of aspen shoot blight infection. Since these features are of concern when attempting to determine disease impact, it is likely that decapitation will become useful to pathologists attempting to quantify the economic impact of *P. americana* infection on *P. tremuloides*. If that is indeed the case, decapitation will join the simulation techniques developed by Lockwood et al. (1977), Zilberstein et al. (1985), and Lusso and Pascholati (1999) in the pathologist's toolbox.

Table 2.1. Origin for host material used in both experiments. Coordinates for *Populus tremuloides* propagule source location are approximate and based on the location of nearest town or city. F indicates the field experiment. GC indicates the growth chamber experiment.

Experiment	Seedlot / Clone Identifier	Nursery	Nursery Location	Approximate Source Location
F	Seedlot 1	Landing Nursery	Vernon, BC	50°N-120°W
F	Seedlot 2	Prairie Farm Rehabilitation Administration	Indian Head, SK	50°N-103°W
F	Seedlot 3	Alberta Nurseries and Seeds Ltd.	Bowden, AB	56°N-111'W
F	Seedlot 4	Jeffries Nurseries Ltd.	Portage-la-Prairie, MB	49°N-98°W
GC	Clone 1	Woodmere Nursery Ltd.	Fairview, AB	56°N-118°W
GC	Clone 2	Woodmere Nursery Ltd.	Fairview, AB	56°N-118°W

Table 2.2. List of isolates of *Pollaccia americana* used in both experiments. F indicates the field experiment. GC indicates the growth chamber experiment. UAMH refers to the University of Alberta Microfungus Collection and Herbarium.

Experiment	Isolate Identifier	Approximate Source Location	Herbarium #
F	PR 6	53°N-118°W	UAMH 9885
F	PR 7	53°N-118°W	
F	PR 10	54°N-113°W	UAMH 9886
F, GC	ASB 3	57°N-119°W	UAMH 9888
F. GC	ASB 9	53°N-115°W	
F	ASB 11	53°N-115°W	UAMH 9889
F	ASB 13	58°N-123°W	UAMH 9890
F, GC	ASB 17	54°N-116°W	UAMH 9891
F, GC	ASB 22	56°N-117°W	UAMH 9892
F, GC	ASB 24	56°N-117°W	

Table 2.3. Composition of conidial suspensions used to inoculate trees. [Conidia] refers to the number of conidia mL^{-1} in suspensions.

Inoculation	Isolates Present	[Conidia]	% Germination
F, Block 1	ASB 3, ASB 9, ASB 11, ASB 17, ASB 22, ASB 24	24×10^4	> 92%
F, Block 2	PR 7, PR 10, ASB 3, ASB 9, ASB 17, ASB 24	48×10^4	> 96%
F, Block 3	PR 6, PR 7, PR 10, ASB 9, ASB 11, ASB 13	44×10^4	> 90%
F, Block 4	PR 6, PR 7, PR 10, ASB 9, ASB 11	31×10^4	> 90%
GC, all trees	ASB 3, ASB 9, ASB 17, ASB 22, ASB 24	18×10^4	> 90%

Table 2.4. Sample size for the field experiment. n refers to the final number of samples trees comprising the mean value for each block-seedlot-injury combination. SLM refers to shoot length measurements. ASC refers to axillary shoot counts.

Injury Treatment	Seedlot	n							
		Block 1		Block 2		Block 3		Block 4	
Measurement type →		SLM	ASC	SLM	ASC	SLM	ASC	SLM	ASC
ASB	1	0	12	4	12	0	12	3	12
	2	5	9	5	7	6	10	3	6
	3	5	5	2	6	6	9	4	7
	4	2	6	3	3	7	8	7	8
Control	1	12	12	11	12	12	12	11	12
	2	10	10	10	12	11	11	12	12
	3	11	11	10	12	11	12	10	10
	4	12	12	12	12	12	12	11	12
Decapitation	1	11	12	10	12	11	11	11	12
	2	11	11	10	10	10	10	11	11
	3	10	10	9	9	11	11	12	12
	4	12	12	10	10	12	12	11	11

Table 2.5. Significance levels associated with random-factor effects on branch length in field-grown trees.

Response Variable	Seedlot (df=3)	Seedlot-by-Injury (df=6)
A1	0.0172	0.2792
A2	0.0084	0.2123
L1	< 0.0001	0.6541
L2	0.1838	0.1302

Table 2.6. Goodness-of-fit statistics for main effects and interactions in the analysis of axillary shoot development among field-grown trees. a) Comparison of diseased and decapitated trees. b) Comparison of control and injured (diseased and decapitated) trees.

a) diseased vs. decapitated			
Effect	**Component L^2**	**df**	**P value**
Injury I_i	0.00	1	1.0000
Seedlot L_k	16.59	3	0.0009
Seedlot-by-Injury Interaction IL_{ik}	0.33	3	0.9543

b) control vs. injured			
Effect	**Component L^2**	**df**	**p value**
Injury I_i	35.98	1	< 0.0001
Seedlot L_k	9.18	3	0.0270
Seedlot-by-Injury Interaction IL_{ik}	0.78	3	0.8542

Table 2.7. Goodness-of-fit statistics for main effects and interactions in the analysis of axillary shoot development among trees grown in the growth chamber. a) Comparison of diseased and decapitated trees. b) Comparison of control and injured (diseased and decapitated) trees.

a) diseased vs. decapitated			
Effect	**Component L^2**	**df**	**p value**
Temperature T_i	0.02	2	0.9512
Injury I_j	3.39	1	0.0656
Temperature-by-Injury Interaction TI_{ij}	1.97	2	0.3734

b) control vs. injured			
Effect	**Component L^2**	**df**	**p value**
Temperature T_i	0.01	2	0.9950
Injury I_j	38.85	1	< 0.0001
Temperature-by-Injury Interaction TI_{ij}	0	2	1.0000

Figure 2.1. Photographs of trembling aspen to demonstrate the placement and result of decapitation. a) Untreated tree and the location of the decapitating cut (arrow). b) Decapitated tree showing the remnants of the shoot apex as a necrotic stub (arrow). Leaves were considered partially expanded and suitable for inclusion in the leaf count if the midrib and veins were visible.

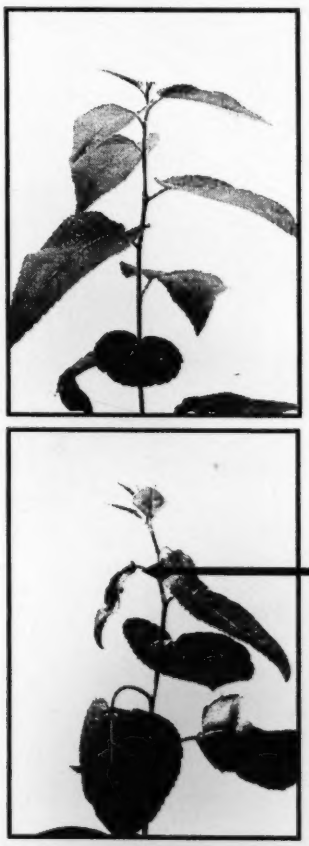
a)

b)

Figure 2.2. Photographs of trembling aspen seedlings showing different levels of ASB infection. a) Healthy tree which has not been inoculated with *P. americana*. b) Tree inoculated with *P. americana* showing lesions localized to one leaf (arrow). c) Tree inoculated with *P. americana* showing a necrotic shoot apex (arrow), and the development of axillary shoots (arrow). Of these three trees, only that shown in c) has sufficient infection to be considered "diseased" for disease incidence and morphology measurements.

a)

b)



c)

Figure 2.3. Diagram of the shoot of a two year old aspen tree showing first order branches. Second order branches on height growth increment (hgi) 2001 and leaves on both hgi's omitted for clarity. All first order branches on the tree were measured and scaled as a proportion of total growth. Branches subject to statistical analysis are identified (arrows). A1 refers to the longest axillary branch, A2 to the second longest axillary branch, L1 to the longest lateral branch, and L2 to the second longest lateral branch. Modified from Wu and Hinckley (2001).

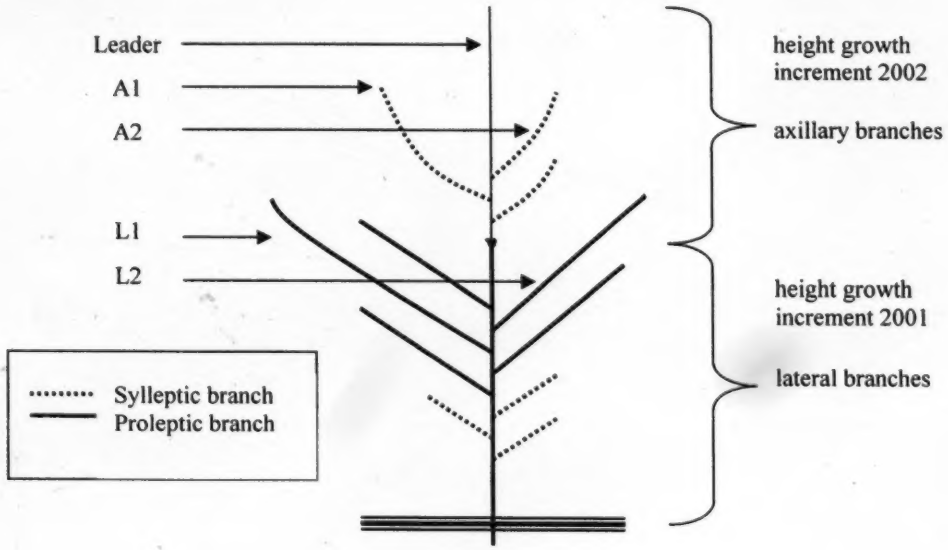


Figure 2.4. Percent of trees with ASB-killed leaders by seedlot and block in the field. Differences among seedlots were significant at $\alpha = 0.05$. n = 12 trees for each block-seedlot combination.

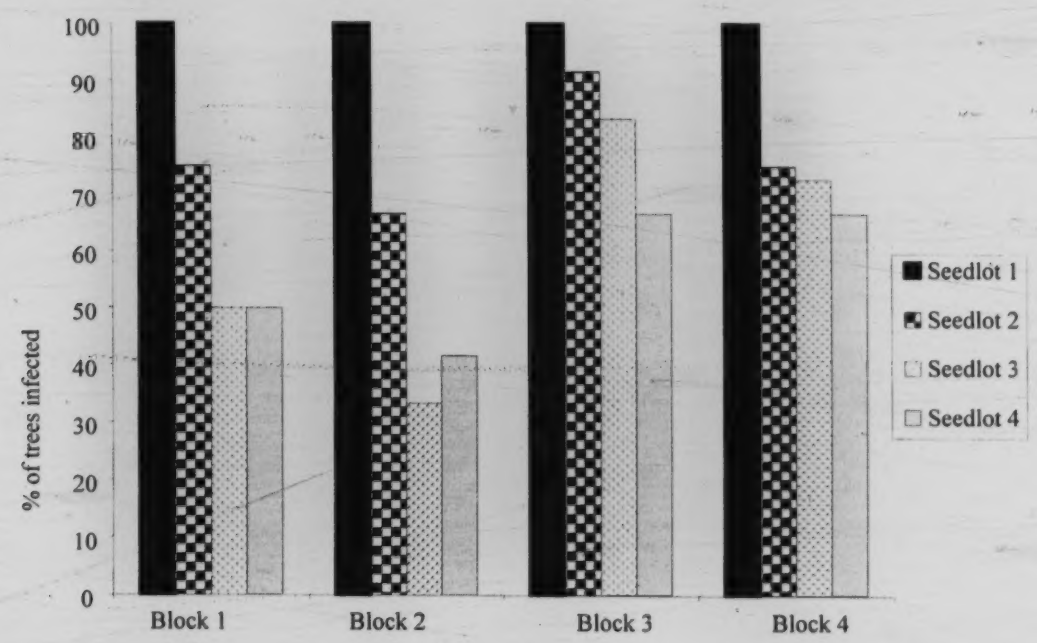


Figure 2.5. Percent of trees with ASB-killed leaders by clone and temperature in the growth chamber. The difference in slope between the two clones was non-significant at $\alpha = 0.05$. n = 6 trees for each combination of clone and temperature.

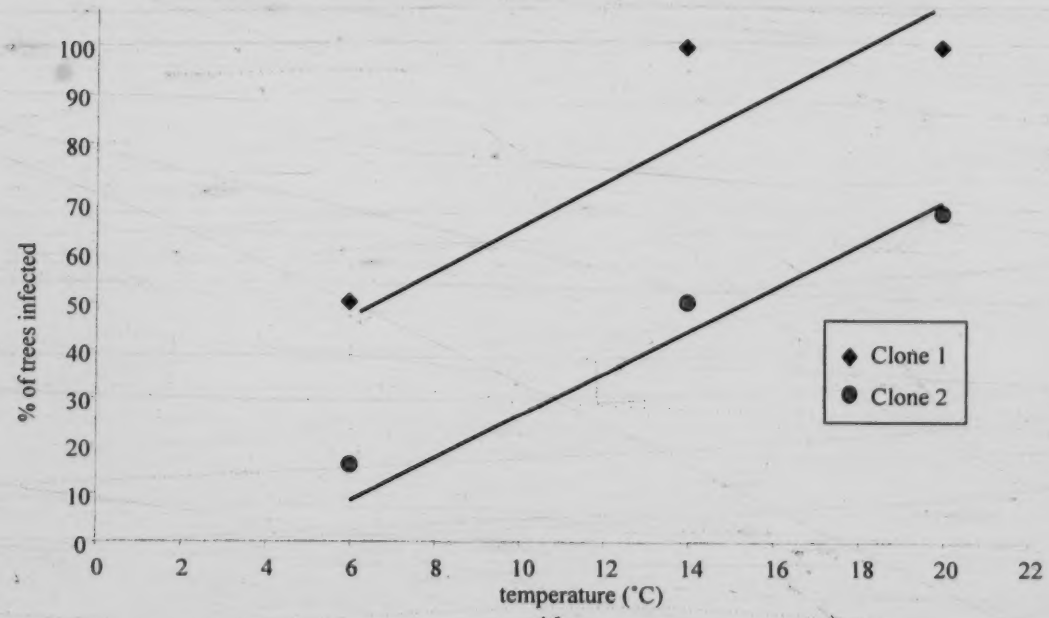
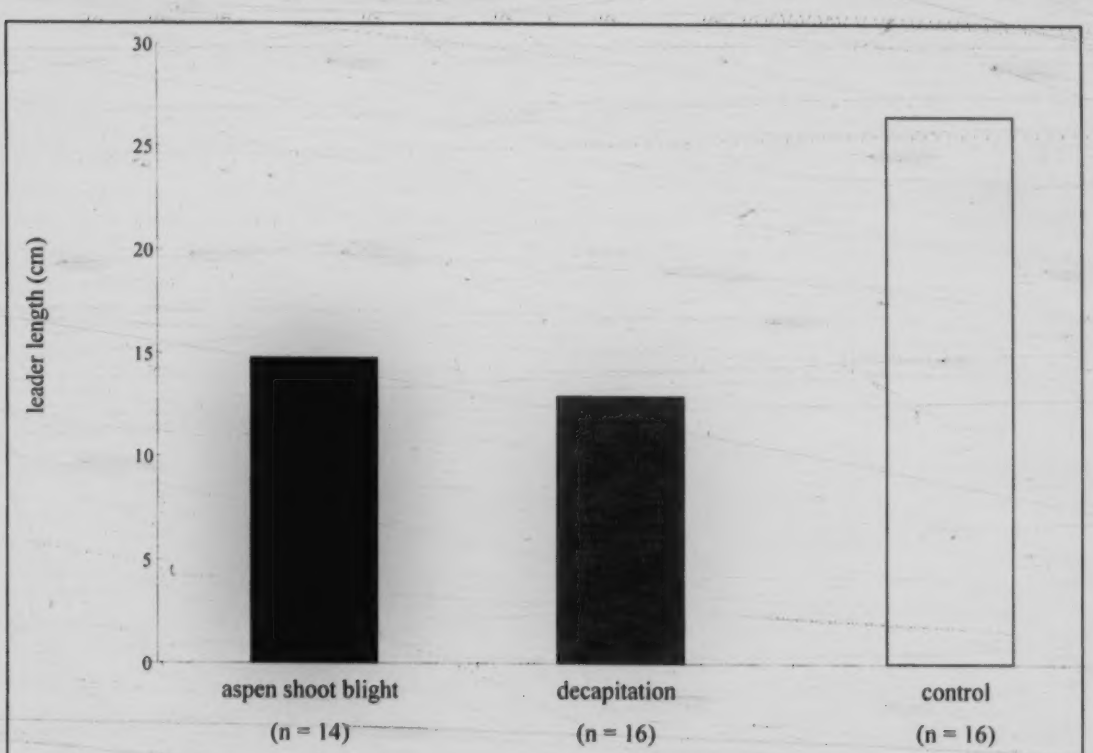
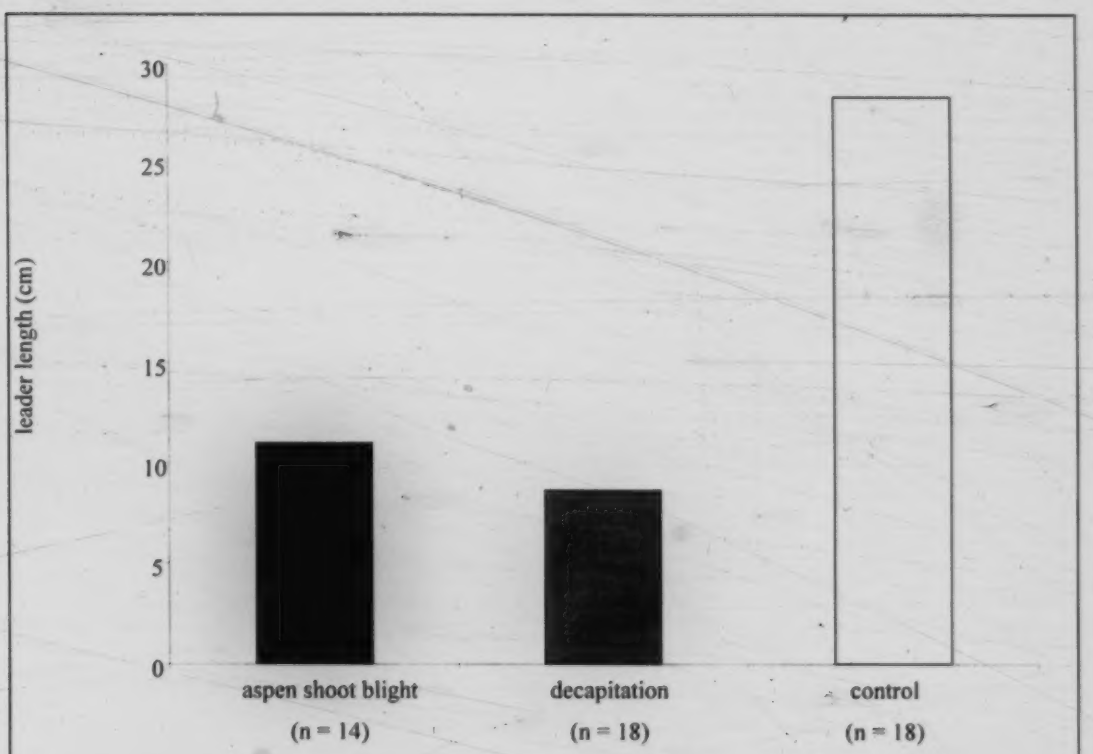


Figure 2.6. Least square means (chart) and statistical contrasts (embedded table) for the effect of injury treatments on leader length in the field. c.i. refers to confidence interval. Sample size (n) indicates the number of block-seedlot-injury combinations used in the calculation of treatment means. Mean values for each block-seedlot-injury combination were calculated from a varying number of sample trees (Table 2.2).



Statistical Comparison (Contrast)	Mean Difference +/- 95% c.i. on the difference	p-value
control vs. injury	12.6 cm +/- 4.2 cm	0.0003
ASB vs. decapitation	1.8 cm +/- 6.9 cm	0.3942

Figure 2.7. Least square means (chart) and statistical contrasts (embedded table) for the effect of injury treatments on leader length in the growth chamber. c.i. refers to confidence interval. Sample size (n) indicates the number of clone-temperature-injury combinations used in the calculation of means. Typically 2 sample trees were used to calculate the mean value for each clone-temperature-injury combination.



Statistical Comparison (Contrast)	Mean Difference +/- 95% c.i. on the difference	p-value
control vs. injury	18.5 cm +/- 3.1 cm	< 0.0001
ASB vs. decapitation	2.4 cm +/- 3.8 cm	0.2153

Figure 2.8. Mean leader lengths of control trees by soil temperature. Overall temperature differences were significant at $\alpha = 0.05$. Error bars indicate 95% confidence intervals. n = 6 for each temperature. Sample size reflects the number of clone-temperature-injury combinations used in the calculation of means. Typically 2 sample trees were used to calculate the mean value for each clone-temperature-injury combination.

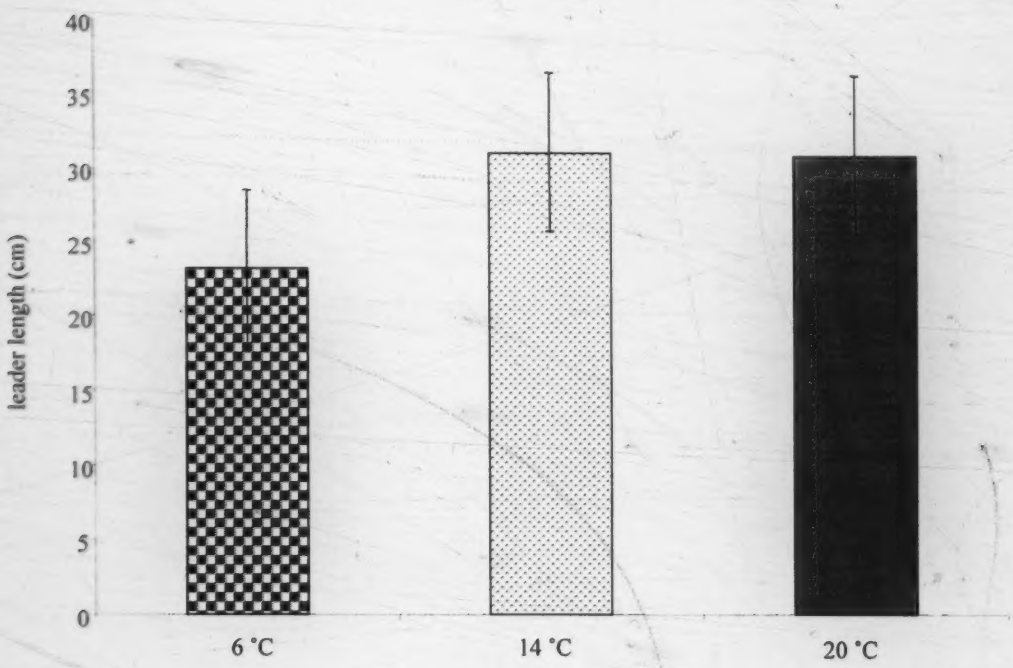


Figure 2.9. Branch growth pattern of field-grown trees. Growth for each branch is expressed as the average proportion of the total growth of each tree. Branches were ordered by length, such that L1 refers to the longest lateral branch and A1 refers to the longest axillary branch on each tree. Sample size (n) indicates the number of block-seedlot-injury combinations used in the calculation of treatment means. Mean values for each block-seedlot-injury combination were calculated from a varying number of sample trees (Table 2.2).

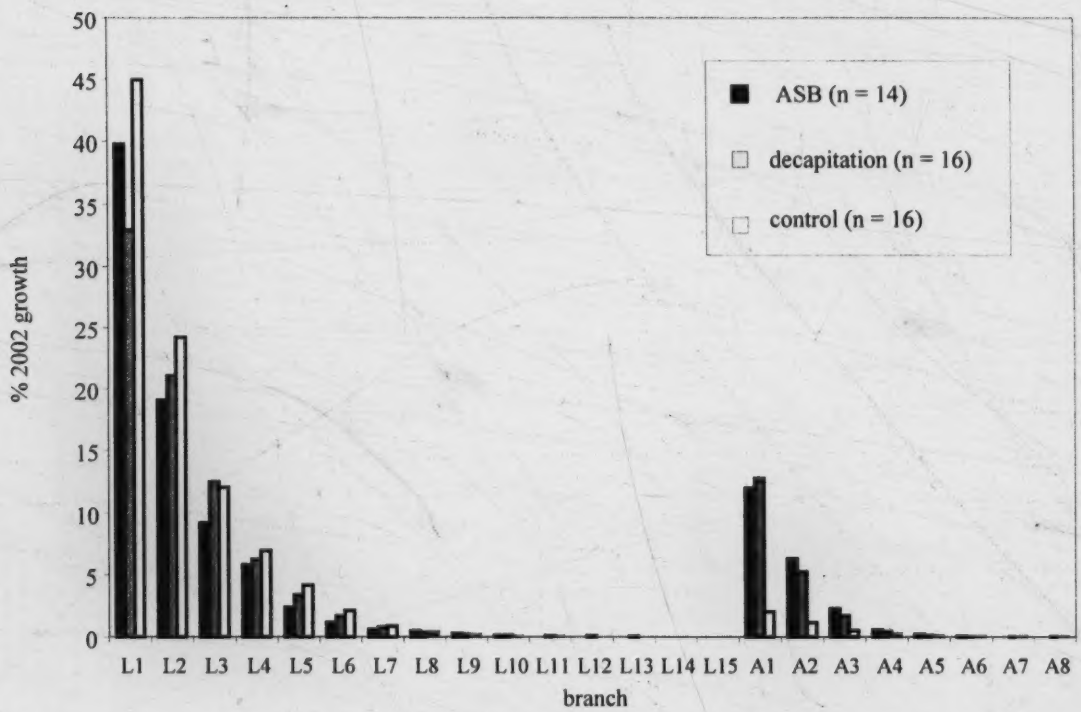
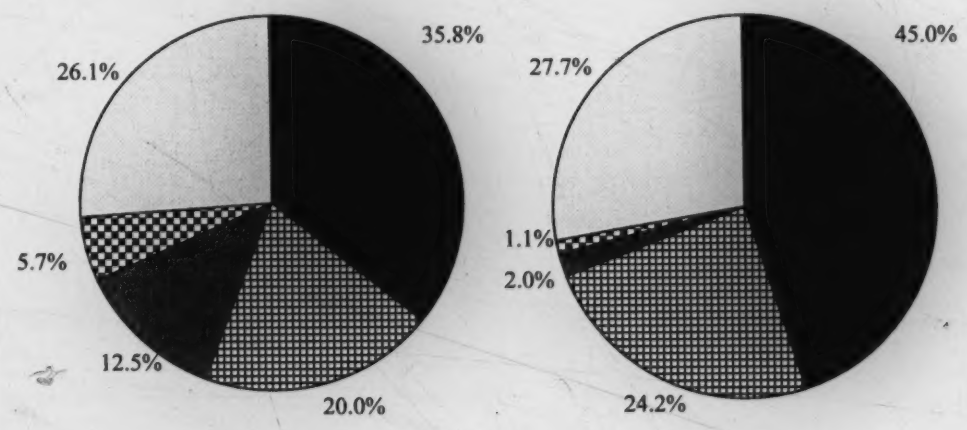


Figure 2.10. Branch growth comparison (chart) and statistical contrasts (embedded table) for injury treatments in the field: injury vs. control. Sample size (n) indicates the number of block-seedlot-injury combinations used in the calculation of treatment means. Mean values for each block-seedlot-injury combination were calculated from a varying number of sample trees (Table 2.2).

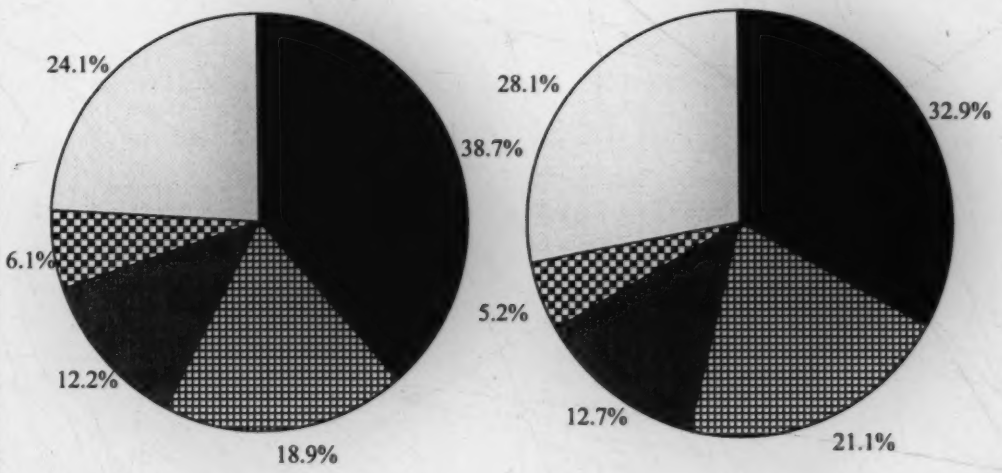


injury (ASB and decapitation)
(n = 30 for each branch)

control
(n = 16 for each branch)

	Branch	Mean of difference +/- 95% c.i.	p value	Holm-adjusted p
	Longest Lateral (L1)	9.1%+/- 7.9%	0.0289	0.0492
	Second Longest Lateral (L2)	4.2% +/- 3.5%	0.0246	0.0492
	Longest Axillary (A1)	10.5% +/- 5.2%	0.0034	0.0102
	Second Longest Axillary (A2)	4.5% +/- 0.2%	0.0020	0.0080
	Other Branches (pooled)	n/a	n/a	n/a

Figure 2.11. Branch growth comparison (chart) and statistical contrasts (embedded table) for injury treatments in the field: ASB vs. decapitation. Sample size (n) indicates the number of block-seedlot-injury combinations used in the calculation of treatment means. Mean values for each block-seedlot-injury combination were calculated from a varying number of sample trees (Table 2.2).



	Branch	Mean of difference +/- 95% c.i.	p value	Holm-adjusted p~
	Longest Lateral (L1)	5.8% +/- 10.7%	0.2660	0.2660
	Second Longest Lateral (L2)	2.1% +/- 4.9%	0.3644	0.3644
	Longest Axillary (A1)	0.55% +/- 7.2%	0.8728	0.8728
	Second Longest Axillary (A2)	0.89% +/- 3.0%	0.5307	0.5307
	Other Branches (pooled)	n/a	n/a	n/a

Figure 2.12. Percent of field-grown trees with axillary branches. The solid portion of each bar represents the percent of trees in which at least one axillary branch was present. The stippled portion represents the percent of trees that did not develop any axillary branches. a) Comparison of diseased and decapitated trees. b) Comparison of control and injured (diseased and decapitated) trees. SL, C, D, and I refer to seedlot, control, decapitation, and injury. Sample sizes (number of trees) are indicated above each bar.

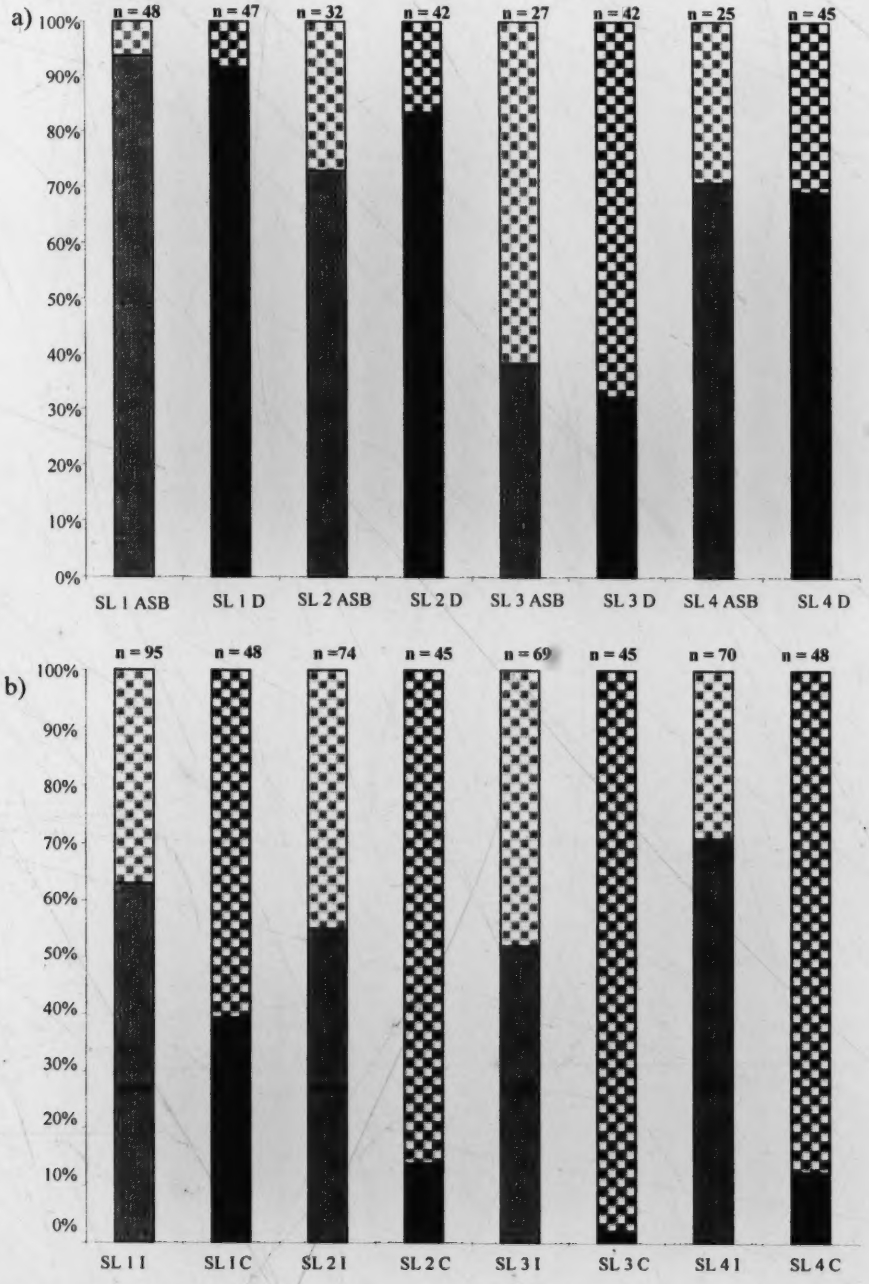


Figure 2.13. Mean number of axillary shoots produced per field-grown tree from each of four seedlots. Differences among seedlots were significant at $\alpha = 0.05$. $n = 4$ for each seedlot-injury combination. Sample size reflects the number of block-seedlot-injury combinations used in the calculation of treatment means. Mean values for each block-seedlot-injury combination were calculated from a varying number of sample trees (Table 2.2).

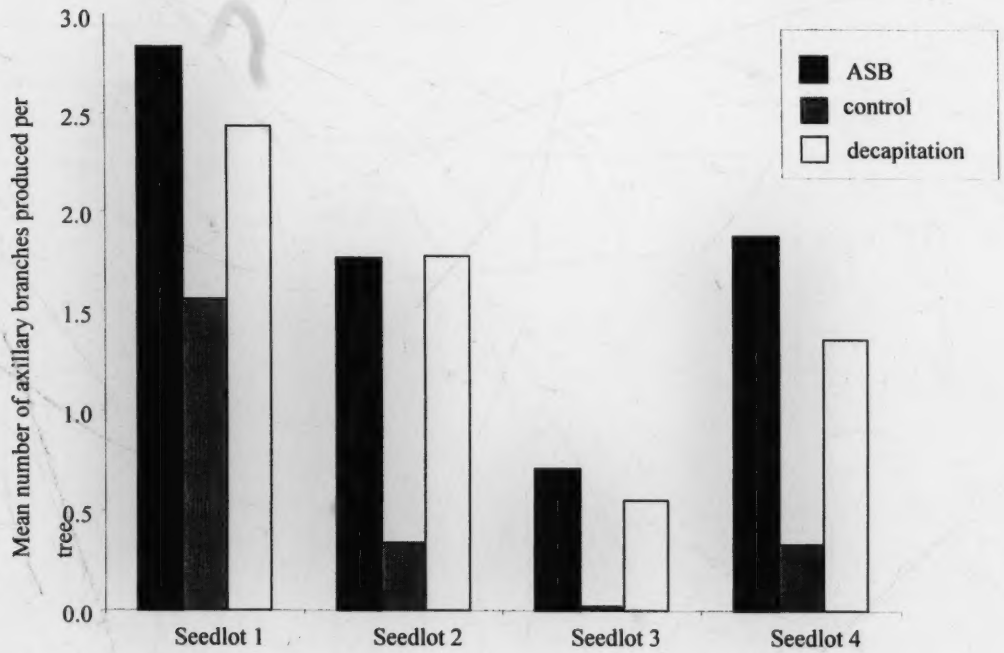
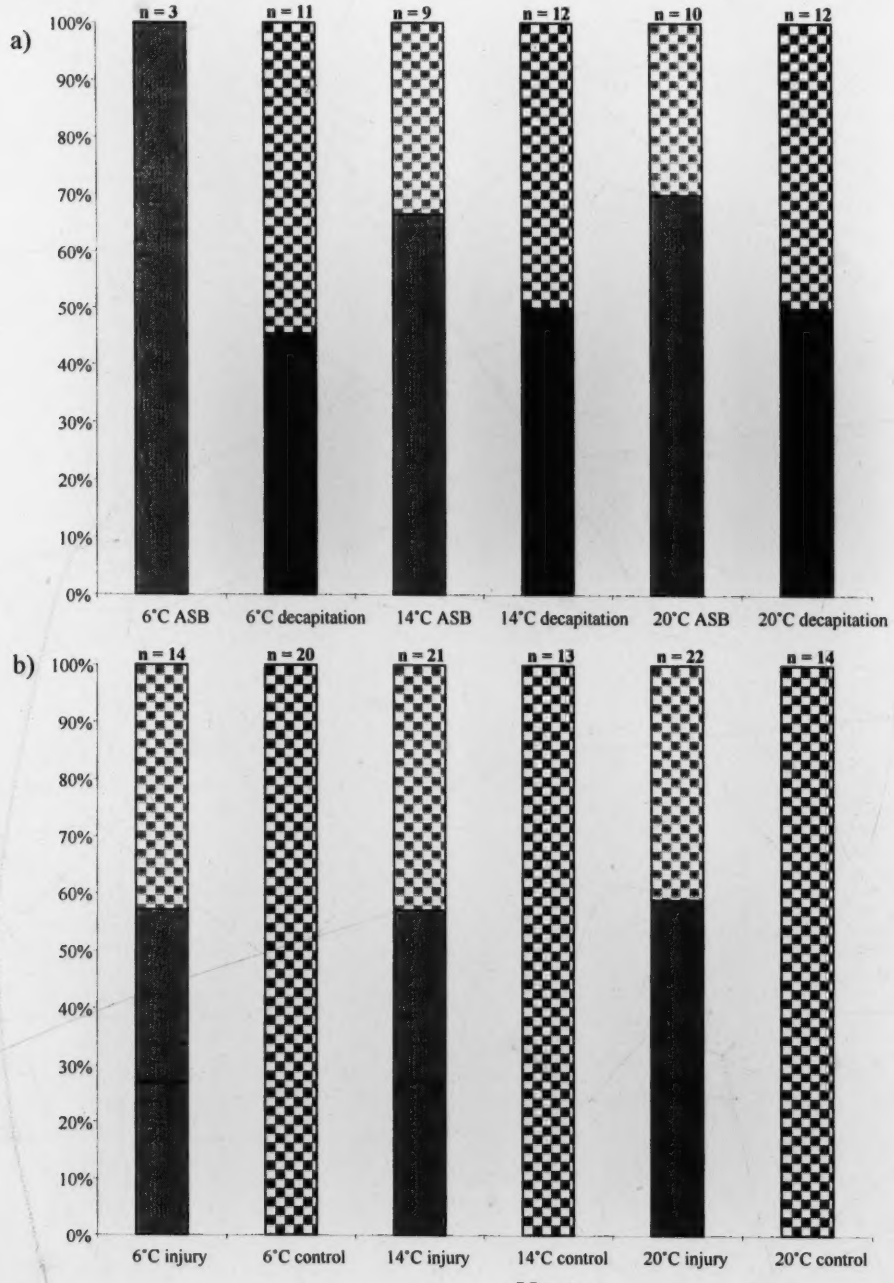


Figure 2.14. Percent of trees in the growth chamber with axillary branches. The solid portion of each bar represents the percent of trees in which at least one axillary branch was present. The stippled portion represents the percent of trees that did not develop any axillary branches. a) Comparison of diseased and decapitated trees. b) Comparison of control and injured (diseased and decapitated) trees. SL, C, D, and I refer to seedlot, control, decapitation, and injury. Sample sizes (number of trees) are indicated above each bar.



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Chapter 3: General Discussion and Conclusions

3.1 Aspen Architecture and Disease Impact

The results of both the field and growth chamber experiments indicate that the most consistent effect of both aspen shoot blight (ASB) and decapitation is a loss of the correlative inhibition of axillary branches (apical dominance). This loss of apical dominance has been related to a loss of "desirable form" as has been suggested in the literature (Anderson and Anderson 1980; Gross and Basham 1981). Understanding the potential impact of a loss of "desirable form", however, requires a more formal understanding of the architecture of trembling aspen (*Populus tremuloides* Michx.).

The study of tree architecture was pioneered by Hallé and Oldeman (1970), whose work grouped the arboreal diversity of tropical rainforests into 23 models based on the configuration and relative proportion of stem, branches, leaves, and sex organs (Hallé et al. 1978). Further revision of these ideas has led to the understanding that the shape of a tree is the result of a combination of deterministic and opportunistic processes: architecture and reiteration, respectively (Tomlinson 1983). These processes account for the genetic predisposition of trees to a particular crown shape, as well as any characteristic responses to environmental factors (e.g. injury) by way of repeating aspects of the original architectural model.

Hallé, Oldeman, and Tomlinson's research is applicable to trees in temperate forests. When this concept of architectural analysis is combined with that based on leaf layer patterns (Horn 1971), number of dominant stems (Zimmerman and Brown 1971), bifurcation ratio (Oohata and Shedei 1971), and ratios of branch and trunk diameter (Stevens and Perkins 1992), a tree's architecture can be unambiguously described. Such work has recently been performed for *P. tremuloides*. Millet et al. (1998, 1999) describe trembling aspen as an example of Rauh's model (Hallé et al. 1978) which has a hierarchic monopodium consisting of one architectural unit which is multilayered (*sensu* Horn 1971) and excurrent (*sensu* Zimmerman and Brown 1971), with a high bifurcation ratio

(*sensu* Oohata and Shedei 1971) and a low branch:trunk diameter ratio (*sensu* Stevens and Perkins 1992). They suggest that such a hierarchical form is adapted to early seral growth (Millet et al. 1999).

Reiteration is a process commonly used by trees to respond to environmental stresses or opportunities (Hallé 1986). In this type of transformation, a tree repeats the architectural unit either by converting an axis from plagiotropy to orthotropy, or by activating resting meristems (Hallé et al. 1978). An architectural unit can be thought of as a group of axes with a hierarchical structure that is delineated from other units by a discontinuity of hierarchy (Millet et al. 1998). In many trees this reiterate is maintained as tree-within-a-tree (Hallé et al. 1978). In some trees, however, the reiterate is not maintained and the previous number of architectural units is conserved (Hallé et al. 1978). Trembling aspen is one of the latter types; it responds to injury by reiteration, although total reiteration (i.e. when more than one architectural unit is maintained) is "virtually non-existent" (Millet et al. 1999, p. 200). Following an injury to the leader, aspen undergoes a transient disorganization among shoots which sets up a competition for dominance between branches. In many cases, one branch dominates and ends the disorganization by imposing apical control; this leads to a restoration of hierarchy and the maintenance of a single architectural unit. This strategy of limited reiteration and strong apical control has most likely developed in order to preserve an uninterrupted hierarchy which promotes rapid trunk growth (Millet et al. 1999).

3.2 Translating Architecture to Impact

3.2.1. Competition

When one considers that aspen is a shade-intolerant early successional species which often grows in high density clonal stands (Peterson and Peterson 1992), a growth strategy which prioritizes height growth seems an appropriate answer to intraspecific (and intra-clonal) ramet competition for light. As competition for light is most likely to be asymmetric (Weiner 1990), a size advantage relative to one's neighbour increases fitness.

Injuries to young aspen caused by ASB and decapitation should be considered in this context.

In a light competitive environment, any loss of current year's growth might result in competition-induced mortality as an indirect effect of shoot blight infection. Such influence of a pathogen on intraspecific competition was shown by Verwijst (1993), who reported high mortality of self-thinning birch (*Salix viminalis* L.) after a leaf rust [*Melampsora epitea* Thüm] epidemic predisposed infected trees to terminal-killing frost. As shoot blight and decapitation both cause the same sort of damage as that reported by Verwijst (1993) (i.e. a loss of leader length), both injuries likely have the potential to influence mortality in highly competitive situations.

This effect might be exacerbated by the loss of apical dominance shown in Chapter 2. A number of ecologists have suggested that apical dominance may be an adaptation of plants to light competition (Aarssen and Irwin 1991, Järemo et al. 1996). This argument was well outlined by Järemo et al.:

> Plants having a rapid height growth effectuated by a few meristems which suppress the development and elongation of lateral shoots are competitively superior under light limited conditions as compared to plants with more branched architecture. Compensatory responses could, therefore, be a by-product of lateral meristems being released from correlative inhibition or apical dominance. Furthermore, the magnitude of the achieved compensatory response when shoot apices are removed may also reflect the competitive environment to which the plant is adapted. Due to the stronger requirement for apical dominance in competitive environments, one may expect stronger compensatory responses in plants adapted to more severe competition for light (Järemo et al. 1996, p. 239).

When viewed from this perspective, a loss of an aspen's form may make it more susceptible to intraspecific light competition. The ultimate impact of this potential competition on aspen ecology, however, would likely be situation-specific.

3.2.1 Resource capture

An injury to the leader which necessitates reiteration may produce a crown which is sub-optimal for light interception if the reiterate is maintained; this can occur even in absence of competition between neighbours. Much work has gone into understanding the role of canopy architecture in providing an efficient photosynthetic leaf arrangement in poplar species. Investigators have tried to understand the environmental factors which influence branching patterns (Marino and Gross 1998; Nelson et al. 1981), the role of branch morphology in biomass production (Burk et al. 1983; Isebrands and Nelson 1982), and the genetic basis for poplar architecture (Wu 1998; Wu and Stettler 1994, 1996). All of this work is based on the idea that phenotypes with superior form (e.g. ideotypes *sensu* Donald 1968) will be more efficient at converting light to biomass and will have found an optimal solution to the trade-off between maximum photosynthetic surface (leaf area) and minimum energy investment (branch volume) (Stevens and Perkins 1992). If aspen utilization is to become more intensive in the future, losses in growth efficiency due to disease-modified crowns will become an important impact.

3.2.2. Bole Formation

Forks, bayonet relays, or shoots with an acute angle of insertion may result from a loss of apical dominance, and may prevent the formation of a straight bole of sufficient length to make its use operationally sound. Provincial ground rules in Alberta define a merchantable deciduous tree as "one that has a minimum stump diameter of 15 cm outside bark and a merchantable length of 4.88 m or greater to a 10 cm top diameter inside bark, or to the point where the stem is unusable or there is no central stem due to heavy branching" (Alberta Environmental Protection 1994). As such, any persistent defects which prevent the production of a straight bole of over 4.88 m long may reduce the utility of a harvested aspen tree. While the effects of form defects on bole formation in aspen have not been well studied, form defects of *Fraxinus excelsior* L. (European ash) have recently been classified according to their severity and likelihood of reducing bole quality (Balandier 1997). These defects included some that disappeared or improved

over time, such as whorls or dominant/co-dominant shoots, and some that were persistent and detrimental to timber quality, such as shoots with an acute angle of origin, bayonet relays, and forks (Table 3.1). Relationships between the terminal and dominant/co-dominant shoots were often transient; the defect improved (branches lost dominance but did not disappear) in 70% and worsened (became acute or forks) in 10% of cases. Forks rarely became innocuous; they became bayonet relays or acute shoots 35% of the time and remained as forks in the remainder of cases.

Though no formal architectural analysis has been performed for *Fraxinus excelsior*, *F. americana* L. (white ash) was shown to have a hierarchical architecture similar to that of aspen, especially when young (Millet et al. 1999). The effect of form defects in ash may therefore provide some indication of the detrimental effects caused by a loss of optimal form via an ASB infection. Based on the loss of apical dominance evident in Chapter 2, one would suspect that ASB infections may result in the initial production of large shoots, dominant/co-dominant shoots, bayonet relays, and/or forks. More research is required to determine whether these branches are maintained, and if so, the proportion that become the more detrimental bayonet relays and forks.

3.3 A List of Unknowns

A considerable amount of space has been dedicated to describing some of the potential impacts of aspen shoot blight, impacts that may arise as a result of leader death and/or the subsequent proliferation of axillary branches. These impacts have been suggested by theory, disease surveys and other field studies, yet none of these potential impacts have been tested experimentally. As such, they remain speculative, and further research is required to accurately quantify potential impacts. This research should take into account four factors which were not addressed in my project: density, severity, timing, and persistence.

3.3.1 Density

Tree form is influenced by density (Marino and Gross 1998; Nelson et al. 1980). Branches in widely spaced trees may grow larger due to increased tree vigour (Balandier 1997). Branches may also be more persistent in open stands due to increased light interception which allows branches to photosynthesize and thus delay the negative feedback process (Larson 1969) that leads to cladopsis (branch shedding). Thus, one would expect that trees in low density plantations may be more susceptible to developing forks after a loss of apical dominance than those in high density stands.

3.3.2 Timing of Wounding

Dance (1961) found that, in Ontario, *Pollaccia* inoculum was most prevalent in June, roughly five to seven weeks following bud flush in *P. tremuloides*. He suggested that the timing of high inoculum availability corresponded to the timing of maximum susceptible tissue availability plus the time required for infection and sporulation (Dance 1961). Assuming a two week infection period, I inoculated trees within the first four weeks of growth to reflect the timing of infection suggested by Dance's work. ASB infections, however, are possible throughout the growing season. As these infections will occur on aspen at different developmental phases, the possibility exists that the impact of infections will change.

Blenis has suggested that this timing effect may result from a differential loss of apical control with growth stage (P.V. Blenis, personal communication). According to this theory, early season leader infections will result in little impact because a lateral shoot quickly becomes the new leader and imposes apical control. Similarly, late season infections will result in no loss of apical control because only the tip of the leader is killed, thus resulting in very little shoot competition and minimal impact. The most damaging losses are expected with mid-season infections, in which a loss of apical dominance sets up a competition between branches for apical control in subsequent years. Research to test this contention is underway.

3.3.3 Severity of Wounding

My research focused on wounding the leader only. This was a simplification from the field situation in which many shoots per tree may become infected by ASB (Anderson and Anderson 1980), but one that in no way jeopardizes my findings; on a shoot-by-shoot basis, the relationship of ASB to decapitation is expected to be consistent because both injuries caused a loss of apical dominance and resulted in the production of shoots from axillary buds. Studies which use decapitation to determine impact should take into account that differences in disease severity exist, and therefore should test multiple scenarios, each corresponding to a different percentage of wounded shoots.

3.3.4 Persistence of Form Defects

Of all the unknown factors, the persistence of form deviations in *P. tremuloides* is probably the most important, and requires further research. Evidence from other species suggests that wounding-induced impacts on tree architecture may be transient. Whitham and Mopper (1985) reported that pinyon pine (*Pinus edulis* Engelm.) crowns which had become shrub-like following chronic herbivory by moth larvae could develop upright crowns when larvae were experimentally excluded for a few years. Researchers studying the effects of moose browsing on Scots pine (*Pinus sylvestris* L.) and birch (*Betula pendula* Roth and *B. pubescens* Ehrh.) suggested that plant morphology recovers approximately 2 to 4 years following actual or simulated herbivory (Danell et al. 1994). Finally, data from pruning studies indicated that forks may not persist in trees with a hierarchic organization plan, such as *F. excelsior* (Table 3.1; Balandier 1997), and may only persist in polyarchic trees, such as silver maple (*Acer saccharinum* L.), when wounds occur at a developmental stage at which the tree is "naturally predisposed" to the production and maintenance of reiterates (Millet and Bouchard 2003, p. 736). As trembling aspen has a hierarchic architecture with limited reiteration (Millet et al. 1999), the persistence of disease-induced form deviations is questionable. Thus, before impacts shown in short-term studies can be extrapolated to the time of stand maturation or harvest, further research to determine the persistence of these impacts is sorely needed.

3.4 Conclusion

Two experiments were conducted in order to compare the morphology of aspen after decapitation and successful infection with *P. americana*; these experiments showed that decapitation reproduced the effects of ASB with fidelity despite significant variation in host populations and in growing conditions. This study had some limitations, (discussed in Chapter 2), but nonetheless provides convincing evidence for the utility of decapitation as an ASB-impact research technique.

The impact of ASB has been suggested by a number of authors to be a loss of current year's growth and change of crown form; both observations were confirmed here. Some potential implications of these short-term impacts have been suggested, and four sources of uncertainty (namely density, timing, severity, and persistence) have been identified; none of them are expected to affect the consistency of the relationship between ASB and decapitation.

My hope is that decapitation will be used as a proxy for ASB in impact studies which test hypotheses based on the foregoing discussion of potential impacts and uncertainties. Such research should be conducted prior to the development of aspen improvement programs which aim to increase the resistance of *P. tremuloides* to *P. americana*. In the context of integrated pest management, ecologically and economically sound decision-making requires an understanding of the biology, beneficial role, and detrimental impact of diseases before deployment of control strategies. As such, decapitation should become a useful research tool for managers of western Canada's abundant aspen resource.

Table 3.1. Description and fate of form defects of 3-6 year old *Fraxinus excelsior*. Defects are listed in order of least to most likely to cause harmful deviations in bole formation. % D refers to the percent of form defects which were not considered a defect in the following year. % I refers to the percent of dominant/co-dominant shoots or forks in which the defect improved by at least one class in the following year. n/a indicates that data were not presented. Modified from Balandier (1997).

Name	Description	% D	% I
large shoot	shoot with diameter ≥ 50% of the main stem	0	n/a
whorl	three or more shoots attached to the main stem within 5 cm	15	n/a
co-dominant or dominant shoot	large shoot (diameter ≥ 50% of the main stem) with an apex at the same level or above that of the main stem	20	70
shoot with an acute angle of origin	shoot with an acute angle of origin which changes the vigour or cylindricity of the main stem	0	n/a
bayonet relay	shoot with break in straightness and/or vigour at a node and with a bayonet form	7	n/a
fork	two shoots with a common insertion and height and diameter of at least 2/3 of that of the other	0	35

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